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FILE 'HOME' ENTERED AT 15:36:56 ON 19 JAN 2001

=> file caplus, biosis, ceaba

| COST IN U.S. DOLLARS | SINCE FILE ENTRY | TOTAL SESSION |
|---|---|---|
| FULL ESTIMATED COST | 1.20 | 1.20 |

FILE 'CAPLUS' ENTERED AT 15:41:27 ON 19 JAN 2001
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=> s plant

L1 944349 PLANT

=> remove duplicates

DUPLICATES IS NOT VALID HERE
The DELETE command is used to remove various items stored by the system.

To delete a saved query, saved answer set, saved L-number list, SDI request, batch request, mailing list, or user-defined cluster, format, or search field, enter the name. The name may include ? . for left, right, or simultaneous left and right truncation.

Examples:

```
  DELETE BIO?/Q           - delete query names starting with BIO
  DELETE ?DRUG/A          - delete answer set names ending with DRUG
  DELETE ?ELEC?/L         - delete L-number lists containing ELEC
  DELETE ANTICOAG/S       - delete SDI request
  DELETE ENZYME/B         - delete batch request
  DELETE .MYCLUSTER       - delete user-defined cluster
  DELETE .MYFORMAT        - delete user-defined display format
  DELETE .MYFIELD         - delete user-defined search field
  DELETE NAMELIST MYLIST - delete mailing list
```

To delete an ordered document or an offline print, enter its number.

Examples:

```
  DELETE P123001C         - delete print request
  DELETE D134002C         - delete document order request
```

To delete an individual L-number or range of L-numbers, enter the L-number or L-number range. You may also enter DELETE LAST followed by a number, n, to delete the last n L-numbers. RENUMBER or NORENUMBER may also be explicitly specified to override the value of SET RENUMBER.

Examples:

```
  DELETE L21              - delete a single L-number
  DELETE L3-L6            - delete a range of L-numbers
  DELETE LAST 4           - delete the last 4 L-numbers
  DELETE L33-             - delete L33 and any higher L-number
  DELETE -L55             - delete L55 and any lower L-number
  DELETE L2-L6 RENUMBER - delete a range of L-numbers and
                            renumber remaining L-numbers
  DELETE RENUMBER         - renumber L-numbers after deletion of
                            intermediate L-numbers
```

Entire sets of saved items, SDI requests, batch requests, user-defined

items, or E-numbers can be deleted.

Examples:

```
  DELETE SAVED/Q - delete all saved queries
  DELETE SAVED/A - delete all saved answer sets
  DELETE SAVED/L - delete all saved L-number lists
  DELETE SAVED   - delete all saved queries, answer sets,
                   and L-number lists
  DELETE SAVED/S - delete all SDI requests
  DELETE SAVED/B - delete all batch requests
  DELETE CLUSTER - delete all user-defined clusters
  DELETE FORMAT  - delete all user-defined display formats
  DELETE FIELD   - delete all user-defined search fields
  DELETE SELECT  - delete all E-numbers
  DELETE HISTORY - delete all L-numbers and restart the
                   session at L1
```

To delete an entire multifile SDI request, enter DELETE and the name of the request. To delete a component from the multifile SDI, enter DELETE and the name of the component.

```
=> file caba, caplus, biosis, ceaba

COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       3.58       4.78

FILE 'CABA' ENTERED AT 15:43:54 ON 19 JAN 2001
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FILE 'CEABA-VTB' ENTERED AT 15:43:54 ON 19 JAN 2001
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=> s plant

L2     1742997 PLANT

=> s cytotoxin

L3        5468 CYTOTOXIN

=> s l2 and l3

L4         104 L2 AND L3

=> s recombinase

L5        3383 RECOMBINASE

=> s l4 and l5

L6           0 L4 AND L5

=> s l2 and l5

L7         124 L2 AND L5
```

```
=> s l7(p)nucleotide

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L30(P)NUCLEOTIDE'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L31(P)NUCLEOTIDE'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L32(P)NUCLEOTIDE'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L33(P)NUCLEOTIDE'
L8          24 L7(P) NUCLEOTIDE#

=> s l7(P)nucleotide

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L30(P)NUCLEOTIDE'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L31(P)NUCLEOTIDE'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L32(P)NUCLEOTIDE'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L33(P)NUCLEOTIDE'
L9          21 L7(P) NUCLEOTIDE

=> d hist


     (FILE 'HOME' ENTERED AT 15:36:56 ON 19 JAN 2001)

     FILE 'CAPLUS, BIOSIS, CEABA-VTB' ENTERED AT 15:41:27 ON 19 JAN 2001
L1        944349 S PLANT

     FILE 'CABA, CAPLUS, BIOSIS, CEABA-VTB' ENTERED AT 15:43:54 ON 19 JAN 2001
L2       1742997 S PLANT
L3          5468 S CYTOTOXIN
L4           104 S L2 AND L3
L5          3383 S RECOMBINASE
L6             0 S L4 AND L5
L7           124 S L2 AND L5
L8            24 S L7(P)NUCLEOTIDE#
L9            21 S L7(P)NUCLEOTIDE

=> d l4 1-10 abs


L4   ANSWER 1 OF 104  CABA COPYRIGHT 2001 CABI
AB   Transport across the nuclear envelope is mediated by transport receptors
     from the Importin beta family. Exportin 1 from Arabidopsis
(AtXPO1/AtCRM1)
     was identified as the nuclear export receptor for proteins carrying
     leucine-rich nuclear export signals (NESs). AtXPO1 shares 42-50% identity
     with its functional homologues from humans and yeasts. AtXPO1 was
     functionally characterised by its interaction with NESs of animal and
     plant proteins, which is inhibited by the cytotoxin
     leptomycin B (LMB), and also by its interaction with the small GTPase
Ran1
     in the yeast two-hybrid system. The existence of a nuclear export pathway
     for proteins in plants was also demonstrated. For the characterisation of
     nuclear export activities, an in vivo assay was established based on the
     localisation equilibrium of a GFP reporter protein fused to both a
nuclear
     localisation signal (NLS) and an NES motif. Using this in vivo assay it
     was demonstrated that the NES of the heterologous protein Rev is also
     functional in plants and that its export is inhibited by LMB. In
addition,
```

a leucine-rich [redacted] in the Arabidopsis protein At[redacted]BP1a was identified. The NES, which [redacted] located at the carboxy terminus [redacted]f the protein, is disrupted by mutating three long chain hydrophobic amino acid residues to alanine (L176A, L179A, V181A). In BY-2 protoplasts the NES of AtRanBP1a is functionally indistinguishable from the Rev NES. It is concluded that these results demonstrate that the machinery for the nuclear export of proteins is functionally conserved in plants.

L4 ANSWER 2 OF 104 CABA COPYRIGHT 2001 CABI
AB The hrp-dependent secretion of two avirulence proteins, AvrBs3 and AvrRxv, by X. campestris pv. vesicatoria [X. vesicatoria] strains that constitutively express hrp genes is reported. Secretion occurred without leakage of a cytoplasmic marker in minimal medium containing BSA, at pH 5.4. Secretion was strictly hrp-dependent because a mutant carrying a deletion in hrcV, a conserved hrp gene, did not secrete AvrBs3 and AvrRxv.
Moreover, the Hrp system of X. campestris pv. vesicatoria was able to secrete proteins from two other **plant** pathogens: PopA, a protein secreted via the Hrp system in Ralstonia solanacearum, and AvrB, an avirulence protein from Pseudomonas syringae pv. glycinea [P. savastanoi pv. glycinea]. Interestingly, X. campestris pv. vesicatoria also secreted YopE, a type III-secreted **cytotoxin** of the mammalian pathogen Yersinia pseudotuberculosis in a hrp-dependent manner. YerA, a YopE-specific chaperone, was required for YopE stability but not for secretion in X. campestris pv. vesicatoria. The results demonstrated the functional conservation of the type III system of X. campestris for secretion of proteins from both **plant** and mammalian pathogens and imply recognition of their respective secretion signals.

L4 ANSWER 3 OF 104 CABA COPYRIGHT 2001 CABI
AB Ribozymes based on a self-splicing group 1 intron that can trans-splice exon sequences into a chosen RNA target to create a functional chimeric mRNA have been designed, and provide a highly specific trigger for gene expression. Ribozymes have been targeted against the coat protein mRNA of a widespread **plant** pathogen, cucumber mosaic cucumovirus. The ribozymes were designed to trans-splice the coding sequence of the diphtheria toxin A chain in frame with the viral initiation codon of the target sequence. Diphtheria toxin A chain catalyses the ADP ribosylation of elongation factor 2 and can cause the cessation of protein translation.
In a Saccharomyces cerevisiae model system, ribozyme expression was shown to specifically inhibit the growth of cells expressing the virus mRNA. A point mutation at the target splice site alleviated this ribozyme-mediated toxicity. Increasing the extent of base pairing between the ribozyme and target dramatically increased specific expression of the **cytotoxin** and reduced illegitimate toxicity in vivo. Trans-splicing ribozymes may provide a new class of agents for engineering virus resistance and therapeutic cytotoxins.

L4 ANSWER 4 OF 104 CABA COPYRIGHT 2001 CABI
AB The effect of cauloside C, isolated from Caulophyllum robustum, on eukaryotic cells was studied. The glycoside acted as a pH-dependent **cytotoxin** and increased K+ leakage and Ca2+ uptake with strong action in acidic media. Cell viability after glycoside action at acidic pH could be recovered by the shift of medium pH from 5.6 to 7.4. Directed transport of low MW effectors such as cAMP and Ca2+ to human embryo fibroblasts under the action of cauloside C has been demonstrated. Ca uptake was accompanied by about a 2-fold stimulation of fibroblast proliferation in serum-free medium. Manifestation of the effect depended on the strictly determined time of the 'open' state of membrane permeability (2 min) and on the concentration of the glycoside in the

medium (1 ng/ml) Cauloside C-stimulated Ca-transport was not blocked by Ca-channel blockers such (including verapamil at 10-6 M), but Ca-channel blockers inhibited cauloside C-stimulated proliferation of fibroblasts. It was concluded that stimulation of fibroblast proliferation was caused by activation of membrane associated Ca-channels. The use of cauloside C as a new biochemical tool for cell permeabilization is suggested.

L4 ANSWER 5 OF 104 CABA COPYRIGHT 2001 CABI
AB Using bioassay-guided fractionation, dioncophylline A was isolated from the leaves and twigs of the tropical liana A. letestui. Dioncophylline A was previously known from Triphyophyllum peltatum and A. abbreviatus. Its in vitro cytotoxicity profile was characterized against the NCI panel of human tumour cell lines.

L4 ANSWER 6 OF 104 CABA COPYRIGHT 2001 CABI
AB The cytotoxicity and antibiotic resistance profiles of A. hydrophila isolates recovered from broiler carcasses and chill water samples taken from a Georgia processing **plant** were determined. Carcasses were sampled at pre- and post-evisceration locations, immediately after immersion chilling and after being boxed, iced and refrigerated for 48 h. Grab samples of chill water were randomly selected for A. hydrophilus recovery. Resistance of isolates to 9 antibiotics was determined with the Bauer disc diffusion method (i.e., to ampicillin, cephalothin, streptomycin, kanamycin, chloramphenicol, naladixic acid [nalidixic acid], tetracycline, neomycin and gentamicin). Multiple antibiotic resistance occurred in 46.2% of 119 isolates. The majority of the multiple antibiotic-resistant isolates (76.4%) were resistant only to ampicillin and cephalothin. The remaining multiple antibiotic-resistant isolates (23.6%) were resistant to various combinations of 2, 3 or 4 antibiotics, most of which were recovered from carcasses immediately after evisceration. **Cytotoxin** activity was detected in 63.8% of all isolates using the Y-1 mouse adrenal tumour cell line. **Cytotoxin** -positive isolates were recovered from all sampling locations including chill water. The highest cytotoxicity titres were shown among isolates recovered from carcasses immediately after evisceration. These data suggest bird faecal contamination as an important source of A. hydrophila in broilers and broiler processing **plant** rather than environmental contamination.

L4 ANSWER 7 OF 104 CABA COPYRIGHT 2001 CABI
AB Ribosomal 5S RNAs were isolated and purified from lupin (Lupinus luteus) seeds and wheat germ and these and the **cytotoxin** alpha -sarcin were used to test a model of secondary and tertiary structures of **plant** 5S rRNAs. alpha -Sarcin is a novel ribonuclease which hydrolyses phosphodiester bonds adjacent to purines in nucleic acids. The digestion pattern obtained for lupin and wheat germ 5S rRNAs strongly suggested the existence of tertiary interactions between residues C34, C35, C36, A37 and G85, G86, G87, U88 as has been previously proposed.

L4 ANSWER 8 OF 104 CABA COPYRIGHT 2001 CABI
AB An account is given of the joint work of **Plant** Genetic Systems of Belgium and R. B. Goldberg's laboratory at UCLA in the USA to engineer tobacco and rape plants with nuclear male sterility. The tapetum-specific promoter from tobacco was fused to 2 ribonuclease (RNase) genes, one a synthesized copy of the gene from Aspergillus oryzae and the other a natural gene encoding the Bacillus amyloliquefaciens RNase. Transformation with one or other of these RNase chimaeras was effected using tobacco tissue which was untransformed or already transformed with a chimaeric reporter gene. Many of the resultant transformants had shrivelled anthers and failed to produce pollen. Female fertility remained normal. Analysis of gene expression in male-sterile anthers showed a marked drop in

expression of t[redacted]tapetum-specific gene at the t[redacted] when the tapetum should have bee[redacted]eveloping, indicating that the [redacted]petum was selectively destroyed as a result RNase expression. Similar results were obtained using oilseed rape.

L4 ANSWER 9 OF 104 CABA COPYRIGHT 2001 CABI
AB Control of ACTH secretion in the pituitary in the absence of target cells for corticotropin-releasing factor (CRF), the most potent ACTH secretagogue, was studied in dissociated bovine anterior pituitary cells treated with a potent selective **cytotoxin**. The **cytotoxin** is a conjugate of the CRF analogue [Nle21,38,Arg36]rat (r) CRF and the **plant** toxin gelonin. Dissociated bovine anterior pituitary cells were pretreated with vehicle, 2 nM ovine CRF, 2 nM cytotoxic conjugate or unconjugated [Nle21,38,Arg36]rCRF and gelonin in amounts equivalent to that of 2 nM cytotoxic conjugate for 12 h, then extensively washed and cultured for 3 days before acute secretion experiments. Unstimulated ACTH secretion was similar in all groups. ACTH secretion in response to CRF was attenuated by pretreatment with the cytotoxic conjugate: CRF (2.5 nM)-stimulated secretion was 7.0, 6.3 and 2.8 times the unstimulated rate in cells pretreated with vehicle, 2 nM CRF or 2 nM cytotoxic conjugate resp. Likewise, the ACTH secretory response to cAMP analogue was attenuated by pretreatment with the conjugate: 8-bromo-cAMP (10 mM)-stimulated secretion was 6.8, 7.1 and 3.3 times the unstimulated rate in cells pretreated with vehicle, CRF or conjugate resp. In contrast, the ACTH responses to vasopressin (VP) or oxytocin (OR) remained intact. VP stimulated the ACTH secretion rate by 4.2, 4.0 and 3.5 times resp. in the 3 groups. OT stimulated the ACTH secretion rate by 2.7, 2.6 and 2.3 times in the 3 groups. Pretreatment with the conjugate attenuated the response to CRF and VP in combination by the same amount as it attenuated the response to CRF alone. The ACTH secretory responses in cells pretreated with unconjugated [Nle21,38,Arg36]rCRF and gelonin were not different from responses in cells pretreated with vehicle. These results suggest that there is a separate mechanism or cell type for OT- and VP-stimulated ACTH secretion distinct from that responsible for the action of CRF on pituitary cells.

L4 ANSWER 10 OF 104 CABA COPYRIGHT 2001 CABI
AB A Shiga-like toxin type II variant (SLT-IIv) is produced by strains of E. coli responsible for oedema disease of swine and is antigenically related to Shiga-like toxin type II (SLT-II) of enterohaemorrhagic E. coli. SLT-IIv is only active against Vero cells, whereas SLT-II is active against both Vero and HeLa cells. The structural genes for SLT-IIv were cloned from E. coli S1191, and the nucleotide sequence was determined and compared with those of other members of the Shiga toxin family. The A subunit genes for SLT-IIv and SLT-II were highly homologous (94%), whereas the B subunit genes were less homologous (79%). The SLT-IIv genes were more distantly related (55 to 60% overall homology) to the genes for Shiga toxin of Shigella dysenteriae type 1 and the nearly identical Shiga-like toxin type I (SLT-I) of enterohaemorrhagic E. coli, (together referred to as Shiga toxin/SLT-I.) The A subunit of SLT-IIv, like those of other members of this toxin family, had regions of homology with the **plant** lectin ricin. SLT-IIv did not bind to galactose- alpha 1-4-galactose conjugated to bovine serum albumin, which is an analog of the eucaryotic cell receptor for Shiga toxin/SLT-I and SLT-II. These findings support the hypothesis that SLT-IIv binds to a different cellular receptor than do other members of the Shiga toxin family but has a similar mode of intracellular action. The organization of the SLT-IIv operon was similar to that of other members of the Shiga toxin family. Iron did not suppress SLT-IIv or SLT-II production, in contrast with its effect on

Shiga toxin/SLT[redacted] Therefore, the regulation of [redacted]thesis of SLT-IIv and SLT-II differs [redacted]m that of Shiga toxin/SLT-I.

=> d hist

(FILE 'HOME' ENTERED AT 15:36:56 ON 19 JAN 2001)

FILE 'CAPLUS, BIOSIS, CEABA-VTB' ENTERED AT 15:41:27 ON 19 JAN 2001
L1 944349 S PLANT

FILE 'CABA, CAPLUS, BIOSIS, CEABA-VTB' ENTERED AT 15:43:54 ON 19 JAN 2001
L2 1742997 S PLANT
L3 5468 S CYTOTOXIN
L4 104 S L2 AND L3
L5 3383 S RECOMBINASE
L6 0 S L4 AND L5
L7 124 S L2 AND L5
L8 24 S L7(P)NUCLEOTIDE#
L9 21 S L7(P)NUCLEOTIDE

=> s l2(P)l5

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L8(P)L23'
L10 97 L2(P) L5

=> d l10 1-10 abs

L10 ANSWER 1 OF 97 CABA COPYRIGHT 2001 CABI
AB The **recombinase** activating genes (RAG1 and RAG2; endonucleases involved in V(D)J recombination) of Japanese pufferfish were isolated, together with a possible homologue of the **plant** 1-aminocyclopropane-carboxylate synthase (ACS) gene, and sequenced. The structure of the RAG genes was identical to that of their homologues from other fish species but the intergenic region was smaller. Reverse transcription-PCR detected RAG transcripts in the kidneys of adult pufferfish.

L10 ANSWER 2 OF 97 CABA COPYRIGHT 2001 CABI
AB We have studied the feasibility in Arabidopsis of using a site-specific recombination system FLP/FRT, from the 2 micro m plasmid of yeast, for making **plant** hybrids. Initially, Arabidopsis plants expressing the FLP site-specific **recombinase** were crossed with plants transformed with a vector containing kanamycin-resistance gene (npt) flanked by FRT sites, which also served to separate the CaMV35S promoter from a promoterless gusA. Hybrid progeny were tested for excision of the npt gene and the positioning of 35S promoter proximal to gusA. GUS activity was observed in the progeny of all crosses, but not in the progeny derived from the self-pollinated homozygous parents. We then induced male sterility in Arabidopsis plants using the antisense expression of a pollen- and tapetum-specific gene, bcp1, flanked by FRT sites. Upon cross-pollination of flowers on the same male-sterile plants with pollen from FLP-containing plants, viable seeds were produced and the progeny hybrid plants developed normally. Molecular analyses revealed that the antisense expression cassette of bcp1 had been excised in these plants. These results show for the first time that a site-specific **recombinase** can be used to restore fertility in male-sterile plants, providing an alternative method for the production of hybrid seeds and plants.

L10 ANSWER 3 OF 97 CABA COPYRIGHT 2001 CABI
AB Recombinant genes conferring resistance to antibiotics or herbicides are widely used as selectable markers in **plant** transformation. Once transgenic material has been selected, the marker gene is dispensable. We report a novel strategy to remove undesirable parts of a transgene after integration into the tobacco genome. This approach is based on the transfer of a vector containing an NPTII gene flanked by two 352 bp attachment P (attP) regions of bacteriophage lambda , and the identification of somatic tissue with deletion events following intrachromosomal recombination between the attP regions. This system was used to delete a 5.9 kb region from a recombinant vector that had been inserted into two different genomic regions. As the attP system does not require the expression of helper proteins to induce deletion events, or a genetic segregation step to remove **recombinase** genes, it should provide a useful tool to remove undesirable transgene regions, especially in vegetatively propagated species.

L10 ANSWER 4 OF 97 CABA COPYRIGHT 2001 CABI
AB A new plasmid series has been created for Agrobacterium-mediated **plant** transformation. The pBECKS2000 series of binary vectors exploits the Cre/loxP site-specific **recombinase** system to facilitate the construction of complex T-DNA vectors. The new plasmids enable the rapid generation of T-DNA vectors in which multiple genes are linked, without relying on the availability of purpose-built cassette systems or demanding complex, and therefore inefficient, ligation reactions. The vectors incorporate facilities for the removal of transformation markers from transgenic plants, while still permitting simple in vitro manipulations of the T-DNA vectors. A 'shuttle' or intermediate plasmid approach has been employed. This permits independent ligation strategies to be used for two gene sets. The intermediate plasmid sequence is incorporated into the binary vector through a plasmid co-integration reaction which is mediated by the Cre/loxP site-specific **recombinase** system. This reaction is carried out within Agrobacterium cells. Recombinant clones, carrying the co-integrative binary plasmid form, are selected directly using the antibiotic resistance marker carried on the intermediate plasmid. This strategy facilitates production of co-integrative T-DNA binary vector forms which are appropriate for either (1) transfer to and integration within the **plant** genome of target and marker genes as a single T-DNA unit; (2) transfer and integration of target and marker genes as a single T-DNA unit but with a Cre/loxP facility for site-specific excision of marker genes from the **plant** genome; or (3) co-transfer of target and marker genes as two independent T-DNAs within a single-strain Agrobacterium system, providing the potential for segregational loss of marker genes.

L10 ANSWER 5 OF 97 CABA COPYRIGHT 2001 CABI
AB Transgenic tobacco plants were produced that contained single-copy pART54 T-DNA with a 35S-uidA gene linked to loxP-flanked kanamycin resistance (nptII) and cytosine deaminase (codA) genes. Retransformation of these plants with pCre1 (containing 35S transcribed cre **recombinase** and hygromycin (hpt) resistance genes) resulted in excision of the loxP-flanked genes from the genome. Phenotypes of progeny from selfed-retransformed plants confirmed nptII and codA excision and integration of the cre-linked hpt gene. To avoid integration of the hpt gene, and thereby generate plants totally free of marker genes, attempts were made to transiently express the cre **recombinase**. Agrobacterium tumefaciens (pCre1) was cocultivated with leaf discs of two pART54-transformed lines and shoots were regenerated in the absence of hygromycin selection. Nineteen of 773 (0.25%) shoots showed tolerance to 5-fluorocytosine (5-fc) which is converted to the toxic 5-fluorouracil by cytosine deaminase. 5-fc tolerance in six shoots was found to be due to

excision of the [redacted]xP-flanked region of the pART5[redacted]-DNA. In four of these shoots excision [redacted]uld be attributed to cre expre[redacted]on from integrated pCre1 T-DNA, whereas in two shoots excision appeared to be a consequence of transient cre expression from pCre1 T-DNA molecules which had been transferred to the **plant** cells but not integrated into the genome. The absence of selectable marker genes was confirmed by the phenotype of the T1 progeny. Thus, through transient cre expression, marker-free transgenic plants were produced without sexual crossing. This approach could be applicable to the elimination of marker genes from transgenic crops which must be vegetatively propagated to maintain their elite genotype.

L10 ANSWER 6 OF 97 CABA COPYRIGHT 2001 CABI
AB Agrobacterium tumefaciens transferred DNA (T-DNA) was targeted to a chromosomally introduced lox site in Arabidopsis thaliana by employing the Cre **recombinase** system. To this end, Arabidopsis target lines were constructed which harboured an active chimaeric promoter-lox-cre gene stably integrated in the **plant** genome. A T-DNA vector with a promoterless lox-neomycin phosphotransferase (nptII) fusion was targeted to this genomic lox site with an efficiency of 1.2-2.3% of the number of random events. Cre-catalysed site-specific recombination resulted in restoration of nptII [encoding kanamycin kinase] expression by translational fusion of the lox-nptII sequence in the integration vector with the transcription and translation initiation sequences present at the target site, allowing selective enrichment on medium containing kanamycin.
Simultaneously, the coding sequence of the Cre **recombinase** was disconnected from these same transcription and translation initiation signals by displacement, aimed at preventing the efficient reversible excision reaction. Of the site-specific recombinants, 89% were the result of precise integration. Furthermore, approx equal to 50% of these integrants were single copy transformants, based on PCR analysis. Agrobacterium T-DNA, which is transferred to **plant** cells as a single-stranded linear DNA structure, is in principle incompatible with Cre-mediated integration. Nevertheless, the results presented here clearly demonstrate the feasibility of the Agrobacterium-mediated transformation system, which is generally used for transformation of plants, to obtain site-specific integration.

L10 ANSWER 7 OF 97 CABA COPYRIGHT 2001 CABI
AB Simple site-specific **recombinase** systems rely on a single-polypeptide **recombinase** to cause a specific reciprocal exchange between 2 short, identical DNA sequences. The 3 systems currently employed in **plant** research are of microbial origin. This paper reviews the most current research which uses site-specific recombination in chromosome manipulation.

L10 ANSWER 8 OF 97 CABA COPYRIGHT 2001 CABI
AB FLP/FRT-mediated site-specific recombination was studied with a recombination-reporter gene system which allows visualization of beta -glucuronidase (GUS) expression after site-specific excisional activation of a silent gusA gene. This system was used for characterization of the functional activity of the Saccharomyces cerevisiae native FLP **recombinase** driven by the cauliflower mosaic caulimovirus (CaMV) 35S promoter (linked to the tobacco mosaic tobamovirus (TMV) omega translational leader) in mediating site-specific recombination of chromosomal FRT sites in tobacco FLP x FRT-reporter hybrids. Six hybrids were generated from crosses of lines containing either a stably integrated recombination-reporter or a FLP-expression construct. The activated gusA

phenotype was specific to hybrid progenies and was not observed in either parental plants or their selfed progenies. Recombination efficiency in whole seedlings was estimated by the percentage of radioactivity on a Southern blot which was incorporated into the recombined DNA product. Estimated efficiency mean values for the six crosses ranged from 5.2 to 52.0%. Histochemical analysis in hybrid plants visualized GUS activity with variable chimaeric patterns and intensities. Recombination efficiency and GUS expression varied both among and within crosses, while higher recombination efficiency coincided with larger and more intense patterns of GUS activity. These data suggest that recombination is induced randomly during somatic developmental stages and that the pattern and intensity generated in a given **plant** are affected by factors imposing variability not only between but also within crosses. Additionally, while recombination in a population of FLP/FRT hybrids may occur in all plants, recombination efficiency may still be low in any given **plant**. The activity of the native, as compared to a modified, FLP in the activation of transgenic traits in tobacco is discussed.

L10 ANSWER 9 OF 97 CABA COPYRIGHT 2001 CABI
AB Several DNA site-specific recombination systems have been shown to function in higher eukaryotic cells. These two-component systems consist of a single-polypeptide **recombinase** and a short recognition sequence of less than 35 bp. Strategic placement of the recognition sites into the **plant** genome has permitted the deletion inversion, integration, and translocation of host and introduced DNA fragments. The use of site-specific recombination for the manipulation of the **plant** genome is reviewed under the following main headings: operability in higher eukaryotes; inversion and deletion of transgenes; insertion of transgenes (mutant lox sites, insertional inactivation of cre, transient expression of cre, and fidelity and copy number of integrated DNA); chromosome rearrangements (chromosomes translocations, chromosome inversions and deletions, and in vitro recombination of chromosomal lox sites); and future prospects (gene regulation and gene transfer, genome engineering, cloning by in vitro reactions, and parasexual transfer of chromosome segments).

L10 ANSWER 10 OF 97 CABA COPYRIGHT 2001 CABI
AB Excision of a DNA segment can occur in Arabidopsis thaliana by reciprocal recombination between 2 specific recombination sites (RSs) when the **recombinase** gene (R) from Zygosaccharomyces rouxii is expressed in the **plant**. To monitor recombination events, several lines of transgenic Arabidopsis plants were generated that carried a cryptic beta -glucuronidase (GUS) reporter gene which was designed in such a way that expression of the reporter gene could be induced by R gene-mediated recombination. Several transgenic lines with an R gene linked to the 35S promoter of cauliflower mosaic caulimovirus were constructed. Each transgenic line carrying the cryptic reporter gene was crossed with each line carrying the R gene. Activity of GUS in F1 and F2 progeny was examined histochemically and recombination between two RSs was analysed by Southern blotting and the polymerase chain reaction. In seedlings and plantlets of F1 progeny and most of the F2 progeny, a variety of patterns of activity of GUS, including sectorial chimaerism in leaves, was observed. A small percentage of F2 individuals exhibited GUS activity in the entire **plant**. This pattern of expression was ascribed to germinal recombination in the F1 generation on the basis of an analysis of DNA structure by Southern blotting. These results indicate that R gene-mediated recombination can be induced in both somatic and germ cells of A. thaliana by cross-pollination of parental transgenic lines.

=> d l10 11-20 abs

L10 ANSWER 11 OF 97 CABA COPYRIGHT 2001 CABI
AB The use of the bacteriophage P1 Cre-lox system for generating conservative site-specific recombination between tobacco chromosomes is described. Two constructs, one containing a promoterless hygromycin-resistance gene preceded by a lox site (lox-hpt) and the other containing a cauliflower mosaic caulimovirus 35S promoter linked to a lox sequence and the cre coding region (35S-lox-cre), were introduced separately into tobacco plants. Crosses between plants harbouring either construct produced plants with the two constructs situated on different chromosomes. Plants with recombination events were identified by selecting for hygromycin resistance, a phenotype expressed upon recombination. Molecular analysis showed that these recombination events occurred specifically at the lox sites and resulted in the reciprocal exchange of flanking host DNA. Progenies of these plants showed 67-100% cotransmission of the new transgenes, 35S-lox-hpt and lox-cre, consistent with the preferential cosegregation of translocated chromosomes. The results illustrated that site-specific recombination systems can be useful tools for the large-scale manipulation of eukaryotic chromosomes in vivo.

L10 ANSWER 12 OF 97 CABA COPYRIGHT 2001 CABI
AB With a view to developing more efficient DNA transformation procedures, yeast FLP/FRT site-specific recombination system has been shown to function in maize and rice protoplasts. FLP **recombinase** activity was monitored by reactivation of beta -glucuronidase (GUS) expression from vectors containing the gusA gene inactivated by insertion of two FRTs (FLP recombination targets) and a 1.31 kb DNA fragment. The stimulation of GUS activity in protoplasts co-transformed with vectors containing FTR-inactivated gusA gene and a chimaeric FLP gene depended on both the expression of the FLP **recombinase** and the presence and structure of the FRT sites. The FLP enzyme could mediate inter- and intramolecular recombination in **plant** protoplasts. These results provide evidence that a yeast recombination system can function efficiently in **plant** cells, and that its performance can be manipulated by structural modification of the FRT sites.

L10 ANSWER 13 OF 97 CABA COPYRIGHT 2001 CABI
AB A general method of gene transfer that does not leave behind a selectable marker in the host genome is described. A luciferase (luc) gene was introduced into the tobacco genome by using the hygromycin phosphotransferase gene (hpt) as a linked selectable marker. Flanked by recombination sites from the bacteriophage P1 Cre/lox recombination system, the hpt gene was subsequently excised from the **plant** genome by the Cre **recombinase**. The Cre-catalysed excision event in the **plant** genome was precise and conservative; without loss or alteration of nucleotides in the recombinant site. After removal of the Cre-encoding locus by genetic segregation, plants were obtained that had incorporated only the desired transgene. Gene transfer without the incorporation of antibiotic-resistance markers in the host genome should ease public concerns over the field release of transgenic organisms expressing such traits. Moreover, it would obviate the need for different selectable markers in subsequent rounds of gene transfer into the same host.

L10 ANSWER 14 OF 97 CABA COPYRIGHT 2001 CABI
AB A mutant Gin **recombinase** of the phage Mu DNA inversion system was successfully expressed in Arabidopsis thaliana and tobacco protoplasts. Site-specific recombination was monitored both physically and

biologically using a recombination assay system in which expression of a beta -glucuronidase (gus) gene requires Gin-mediated recombination. The wild-type Gin protein was not able to promote recombination in **plant** protoplasts, presumably because **plant** cells do not contain a protein that can substitute for the Escherichia coli FIS protein needed for full activity of wild-type Gin in E. coli. A FIS-independent Gin mutant protein, however, was efficient in promoting recombination on recombination substrates introduced transiently and on substrates stably integrated into the **plant** genome. The advantages this system can provide for genetic manipulation of **plant** cells are discussed.

L10 ANSWER 15 OF 97 CABA COPYRIGHT 2001 CABI
AB **Recombinase** encoded by the R gene of pSR1 of the yeast Z. rouxii mediates reciprocal recombination between 2 specific recombination sites (RSs) to induce excision or inversion of the DNA segment that is flanked by the RSs. To monitor the recombination events in tobacco cells, 2 types of cryptic beta -glucuronidase reporter gene were constructed in such a way that recombination such as inversion of the construct or excision of the intervening sequence results in their expression. When these cryptic reporter constructs were transiently introduced together with the R gene by electroporation into protoplasts of tobacco cells, beta -glucuronidase activity was detected. The cryptic reporter genes, when stably resident in the chromosome of tobacco cells, were also activated by the R gene. Structural analyses of the genomic DNA isolated from these tobacco cells showed that the R protein did in fact catalyse precise recombination between 2 copies of RSs in tobacco cells, with resultant activation of the cryptic reporter genes. This observation provides the basis for development of a DNA technology whereby large regions of DNA can be manipulated in **plant** chromosomes. Potential uses of this recombination system are discussed.

L10 ANSWER 16 OF 97 CABA COPYRIGHT 2001 CABI
AB A transient assay demonstrated that the bacteriophage P1 cre gene can be expressed as a functional **recombinase** in tobacco cells. Upon expression in tobacco protoplasts, the Cre protein recognizes its target sites, lox, and mediates reciprocal genetic crossovers at these sites. When the lox sites are present in cis to one another and arranged in either direct or inverted orientations, Cre/lox-specific deletion and inversion events were observed, respectively. The placement of lox sites in trans resulted in the co-integration of the substrates by Cre-mediated intermolecular recombination. It is suggested that the Cre/lox site-specific recombination system might be further developed as an additional tool for manipulating DNA in **plant** cells. Applications relevant to the genetic engineering of higher plants (such as targetting inserted DNA, deleting sequences or generating chromosomal translocations) are discussed.

L10 ANSWER 17 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB Eleven independent GUS-pos. hairy roots were induced by co-cultivation of leaf explants of Antirrhinum majus L. with Agrobacterium tumefaciens strain GV2260 contg. the rol type MAT vector pNPI702. The MAT vector pNPI702 possesses a GUS gene under the 35 S promoter and a removal element in which the 7.6-kb DNA fragments contg. the rolA, B, C and D genes and **recombinase** gene with a 35 S promoter are located between two directly oriented recombination site sequences. A total of 326 adventitious shoots regenerated from 11 independent hairy root lines cultured on 1/2MS medium without **plant** growth regulators at 25 .degree.C under a 16/8 h (day/night) photoperiod after 8 wk of stock-culture of hairy roots and 4 wk of culture of the green segments of hairy roots. Regenerated plants showed either a normal or dwarf morphol.

GUS activity was obsd. in the hairy roots and regenerated shoots. The presence of the GUS gene in the regenerated, morphol. normal plants was confirmed by PCR anal.

L10 ANSWER 18 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB We have created a DNA construct, TREGED (transposon-and **recombinase**-mediated genome deletion), that will automatically induce deletions in **plant** genomes. TREGED contains the maize Ac/Ds transposon, the yeast R-RS site-specific recombination system, the bacterial tetR repression systems, a novel artificial superintron, and the marker genes GUS and Lc. The novelty of TREGED is that only one cross is required to trigger a sequence of events leading to deletion and, simultaneously, to a color assay to detect the deletion. Crossing is done to introduce Ac transposase which activates Ds transposition from TREGED to a nearby chromosome region. Ds transposition, in turn, activates recombination between an engineered RS site on TREGED and an RS site on the transposed Ds fragment, thus deleting the genome segment between TREGED and Ds. The recombination event also deletes Lc or GUS and part of tetR, which triggers expression of GUS or Lc color genes for an upstream or downstream deletion resp. Each TREGED insertion site will produce multiple kinds of deletions identifiable by inspecting a single F1 **plant** and its progeny for colored tissue. The color markers can also be used to differentiate between deletion and other more rare events such as translocation and inversion. We anticipate TREGED will greatly simplify the steps required to obtain useful deletions - eventually allowing the creation of detailed deletion libraries. Such libraries will be particularly useful for anal. of gene and chromatin function in **plant** species with large genomes.

L10 ANSWER 19 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB This invention relates to cDNAs encoding corn and wheat gene RAD51 recombinases. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of the recombination protein, in sense or antisense orientation, wherein expression of the chimeric gene results in prodn. of altered levels of the recombination protein in a transformed host cell. Also disclosed is use of the chimeric gene for pos. selection of transformed plant cells.

L10 ANSWER 20 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB The present invention is directed to isolated promoter sequences from seed-specific genes, such as KNAT411 . When operably linked to either the coding sequence of a heterologous gene or a sequence complementary to a native **plant** gene, the subject promoters direct expression of the coding sequence or complementary sequence in a **plant** seed, including the early embryo. The promoter sequences are useful in expression cassettes and expression vectors for the transformation of plants. Also provided are methods of directing seed-specific expression of a gene or sequence complementary to a native **plant** gene by introducing into a **plant** cell an isolated nucleic acid comprising a subject promoter operably linked to said gene or complementary sequence. Methods for activating a site-specific recombination system in the early embryo of a seed by transforming a **plant** with an expression cassette comprising a subject promoter operably linked to a **recombinase** gene are also provided. Thus, the A. thaliana KNAT411 gene promoter was cloned and sequenced. This gene was found to be active very early in embryogenesis, much earlier than other known seed-specific promoters. Southern anal. indicated that there was only one KNAT411 gene, but there were several KNAT411-like sequences

in the A. thaliana genome. The KNAT411 gene was detd. to have five exons sepd. by four introns. The obsd. position of the third intron (inside the ELK domain) and of the fourth intron (interrupting the homeodomain) is characteristic of knotted genes.

=> d l10 21-30 abs

L10 ANSWER 21 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB The Agrobacterium VirB/D4 transport system mediates the transfer of a nucleoprotein T complex into **plant** cells, leading to crown gall disease. In addn., several virulence proteins must somehow be transported to fulfill a function in planta. Fusions between Cre **recombinase** and VirE2 or VirF were used to directly demonstrate protein translocation into **plant** cells. Transport of the proteins was monitored by a Cre-mediated in planta recombination event resulting in a selectable phenotype and depended on the VirB/D4 transport system but did not require transferred DNA.

L10 ANSWER 22 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB Disclosed are variants of Cre recombinase that have broadened specificity for the site of recombination. Specifically, the disclosed variants mediate recombination between sequences other than the loxP sequence and other lox site sequences on which wild type Cre recombinase is active. In general, the disclosed Cre variants mediate efficient recombination between lox sites that wild type Cre can act on (referred to as wild type lox sites), between variant lox sites not efficiently utilized by wild type Cre (referred to as variant lox sites), and between a wild type lox site and a variant lox site. Also disclosed are methods of recombining nucleic acids using the disclosed Cre variants. For example, the disclosed Cre variants can be used in any method or technique where Cre recombinase (or other, similar recombinases such as FLP) can be used. In addn., the disclosed Cre variants allow different alternative recombinations to be performed since the Cre variants allow much more efficient recombination between wild type lox sites and variant lox sites. Control of such alternative recombination can be used to accomplish more sophisticated sequential recombinations to achieve results not possible with wild type Cre recombinase.

L10 ANSWER 23 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB We have studied the feasibility in Arabidopsis of using a site-specific recombination system FLP/FRT, from the 2.mu.m plasmid of yeast, for making **plant** hybrids. Initially, Arabidopsis plants expressing the FLP site-specific **recombinase** were crossed with plants transformed with a vector contg. kanamycin-resistance gene (npt) flanked by FRT sites, which also served to sep. the CaMV35S promoter from a promoterless gusA. Hybrid progeny were tested for excision of the npt gene and the positioning of 35S promoter proximal to gusA. GUS activity was obsd. in the progeny of all crosses, but not in the progeny derived from the self-pollinated homozygous parents. We then induced male sterility in Arabidopsis plants using the antisense expression of a pollen- and tapetum-specific gene, bcp1, flanked by FRT sites. Upon cross-pollination of flowers on the same male-sterile plants with pollen from FLP-contg. plants, viable seeds were produced and the progeny hybrid plants developed normally. Mol. analyses revealed that the antisense expression cassette

of bcp1 had been excised in these plants. These results show for the first time that site-specific **recombinase** can be used to restore fertility in male-sterile plants, providing an alternative method for the prodn. of hybrid seeds and plants.

L10 ANSWER 24 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB Recombinant genes conferring resistance to antibiotics or herbicides are widely used as selectable markers in **plant** transformation. Once transgenic material has been selected, the marker gene is dispensable. We report a novel strategy to remove undesirable parts of a transgene after integration into the tobacco genome. This approach is based on the transfer of a vector contg. a NPTII gene flanked by two 352 bp attachment P (attP) regions of bacteriophage .lambda., and the identification of somatic tissue with deletion events following intrachromosomal recombination between the attP regions. This system was used to delete a 5.9 kb region from a recombinant vector that had been inserted into two different genomic regions. As the attP system does not require the expression of helper proteins to induce deletion events, or a genetic segregation step to remove **recombinase** genes, it should provide a useful tool to remove undesirable transgene regions, esp. in vegetatively propagated species.

L10 ANSWER 25 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB The **recombinase** activating genes (RAG1 and RAG2) encode nuclear proteins that directly mediate the mechanism V(D)J recombination process that occurs in T- and B-lymphocytes. The expression of RAG1 and RAG2 is required for the proper development of maturing lymphocytes. To identify evolutionary conserved regulatory regions adjacent to both genes we isolated and sequenced a cosmid clone contg. 43 kb of genomic DNA of the Japanese pufferfish, Fugu rubripes. Fugu has a haploid genome of 400 Mb and contains the same no. of genes as the genome of higher vertebrates. With low abundance of repetitive DNA, the genome of the pufferfish has shown to be ideal for comparative genomics. We found three complete genes, RAG1, RAG2 and ACS (a possible homolog of the **plant** 1-aminocyclopropane-carboxylate synthase gene). There is also the 5' exon of a prohormone convertase gene, possibly PACE4. The genetic structure of both RAG1 and RAG2 is identical to that found in other fish, but the size of the intergenic region is smaller in Fugu. Expression anal. by RT-PCR shows the presence of RAG transcripts in kidney of adult Fugu. The human ACS was identified in a cosmid assigned to chromosome 11p11, which is close to the location of the RAGs (11p12). This indicates conservation of linkage between human and pufferfish.

L10 ANSWER 26 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB The present invention describes methods for site-directed integration of a transgene in a **plant** genome using site-specific recombination and site-specific **recombinase** systems. The invention discloses the use of two different **recombinase** recognition sites, in particular two **recombinase** recognition sites which differ in their spacers. The **recombinase** recognition sites usually comprise two inverted repeats of about 13-bp sepd. by a spacer of 8-bp. The active **recombinase** is either provided to the **plant** as an active protein or as a translatable mRNA. Different combinations of **recombinase** recognition sites for integration were employed to ensure a stable chromosomal locus after transgene integration. Modified FRT sites are also provided. Position effect variegation is avoided by this technique. The invention also relates to transgenic plants obtained by a method of the present invention and to their progeny for eventual crop improvement.

L10 ANSWER 27 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB Transgenic tobacco plants were produced that contained single-copy pART54 T-DNA, with a 35S-uidA gene linked to loxP-flanked kanamycin resistance (nptII) and cytosine deaminase (codA) genes. Retransformation of these plants with pCre1 (contg. 35S transcribed cre **recombinase** and hygromycin (hpt) resistance genes) resulted in excision of the loxP-flanked genes from the genome. Phenotypes of progeny from selfed-retransformed plants confirmed nptII and codA excision and integration of the cre-linked hpt gene. To avoid integration of the hpt gene, and thereby generate plants totally free of marker genes, the authors attempted to transiently express the cre **recombinase**. Agrobacterium tumefaciens (pCre1) was cocultivated with leaf disks of two pART54-transformed lines and shoots were regenerated in the absence of hygromycin selection. Nineteen of 773 (0.25%) shoots showed tolerance to 5-fluorocytosine (5-fc) which is converted to the toxic 5-fluorouracil by cytosine deaminase. 5-fc tolerance in six shoots was found to be due to excision of the loxP-flanked region of the pART54 T-DNA. In four of these shoots excision could be attributed to cre expression from integrated pCre1 T-DNA, whereas in two shoots excision appeared to be a consequence of transient cre expression from pCre1 T-DNA mols. which had been transferred to the **plant** cells but not integrated into the genome. The absence of selectable marker genes was confirmed by the phenotype of the T1 progeny. Therefore, through transient cre expression, marker-free transgenic plants were produced without sexual crossing. This approach could be applicable to the elimination of marker genes from transgenic crops which must be vegetatively propagated to maintain their elite genotype.

L10 ANSWER 28 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB The invention relates to methods and compns. for site-specific **recombinase**-mediated mobilization of viral replicons and assocd. DNAs of interest from T-DNA. The methods of the invention comprise Agrobacterium-mediated transfer **recombinase**. The nucleic acids of the invention may addnl. contain expression cassettes encoding the cognate site-specific **recombinase** for the target sites flanking the viral genome. The compns. of the invention further comprise Agrobacterium contg. the nucleic acids of the invention. The compns. and methods of the invention have use in increasing the efficiency of agroinfection, providing high copy nos. of a DNA of interest for transient expression or for integration into a **plant** chromosome, and in simplifying the construction and stable maintenance of vectors for agroinfection and transformation.

L10 ANSWER 29 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB Claimed are compns. and methods for introducing nucleotide sequences at preferred genomic target sites in a eukaryotic genome, particularly a **plant** genome, said compns. comprising transfer cassettes which are flanked by nonhomologous recombination sites. The method involves transforming eukaryotic cells contg. novel non-identical target sites utilizing non-integrating transformation methods. The method results in efficient integration of nucleotides into predetd. genetic locations via the action of a suitable **recombinase** and eliminates random DNA integration. The method is demonstrated in the use of Agrobacterium vectors for the transformation of corn.

L10 ANSWER 30 OF 97 CAPLUS COPYRIGHT 2001 ACS
AB Compns. and methods for introducing DNA of interest into a genomic target site are provided. involving the use of a combination of target sites for two site specific recombinases and expression of a chimeric **recombinase** with dual target site specificity. Thus, the compns.

comprise novel [redacted]e-specific recombinases with sp[redacted]ificities to multiple target sites, a[redacted]nucleotide sequences and expre[redacted]n cassettes encoding these recombinases or target sites. The methods involve transforming a eukaryotic cell having target sites for the novel **recombinase** with a DNA of interest that is flanked by corresponding target sites. Expression of the **recombinase** results in integration of the DNA of interest into the genome of the cell. The compns. and methods of the invention have use in the construction of stably transformed eukaryotic cells, and in particular, **plant** cells. The methods result in the efficient targeted genomic integration of DNA by site-specific recombination.

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=> d hist

     (FILE 'HOME' ENTERED AT 15:36:56 ON 19 JAN 2001)

     FILE 'CAPLUS, BIOSIS, CEABA-VTB' ENTERED AT 15:41:27 ON 19 JAN 2001
L1        944349 S PLANT

     FILE 'CABA, CAPLUS, BIOSIS, CEABA-VTB' ENTERED AT 15:43:54 ON 19 JAN 2001
L2       1742997 S PLANT
L3          5468 S CYTOTOXIN
L4           104 S L2 AND L3
L5          3383 S RECOMBINASE
L6             0 S L4 AND L5
L7           124 S L2 AND L5
L8            24 S L7(P)NUCLEOTIDE#
L9            21 S L7(P)NUCLEOTIDE
L10           97 S L2(P)L5

=> s zea(W)mays

L11      134909 ZEA(W) MAYS

=> s l2 and l11

L12       54532 L2 AND L11

=> s l12 and l5

L13           2 L12 AND L5

=> d l13 all abs


L13  ANSWER 1 OF 2  CABA COPYRIGHT 2001 CABI
AN   93:105419  CABA
DN   931641292
TI   Activity of yeast FLP recombinase in maize and rice protoplasts
AU   Lyznik, L. A.; Mitchell, J. C.; Hirayama, L.; Hodges, T. K.
CS   Department of Botany and Plant Pathology, Purdue University, West
     Lafayette, IA 47907, USA.
SO   Nucleic Acids Research, (1993) Vol. 21, No. 4, pp. 969-975. 37 ref.
     ISSN: 0305-1048
DT   Journal
LA   English
AB   With a view to developing more efficient DNA transformation procedures,
     yeast FLP/FRT site-specific recombination system has been shown to
     function in maize and rice protoplasts. FLP recombinase activity
     was monitored by reactivation of beta -glucuronidase (GUS) expression
from
     vectors containing the gusA gene inactivated by insertion of two FRTs
(FLP
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recombination ta[redacted]ts) and a 1.31 kb DNA fragment[redacted]he stimulation of GUS activity in pro[redacted]asts co-transformed with vecto[redacted] containing FTR-inactivated gusA gene and a chimaeric FLP gene depended on both the expression of the FLP **recombinase** and the presence and structure of the FRT sites. The FLP enzyme could mediate inter- and intramolecular recombination in **plant** protoplasts. These results provide evidence that a yeast recombination system can function efficiently in **plant** cells, and that its performance can be manipulated by structural modification of the FRT sites.

CC FF020 Plant Breeding and Genetics; WW000 Biotechnology; ZZ900 Techniques and Methodology

BT Zea; Gramineae; Cyperales; monocotyledons; angiosperms; Spermatophyta; plants; Oryza

CT Maize; Rice; Biotechnology; Genes; recombination; genetic transformation; techniques; genetics

ORGN **Zea mays**; Oryza sativa; plants; Oryza

AB With a view to developing more efficient DNA transformation procedures, yeast FLP/FRT site-specific recombination system has been shown to function in maize and rice protoplasts. FLP **recombinase** activity was monitored by reactivation of beta -glucuronidase (GUS) expression from vectors containing the gusA gene inactivated by insertion of two FRTs (FLP recombination targets) and a 1.31 kb DNA fragment. The stimulation of GUS activity in protoplasts co-transformed with vectors containing FTR-inactivated gusA gene and a chimaeric FLP gene depended on both the expression of the FLP **recombinase** and the presence and structure of the FRT sites. The FLP enzyme could mediate inter- and intramolecular recombination in **plant** protoplasts. These results provide evidence that a yeast recombination system can function efficiently in **plant** cells, and that its performance can be manipulated by structural modification of the FRT sites.

```
=> s activator(W)transposase

L14          28 ACTIVATOR(W) TRANSPOSASE

=> s l14(P)l11

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L68(P)L53'
L15           0 L14(P) L11

=> s l11(P)l14

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L53(P)L68'
L16           0 L11(P) L14

=> d l14 1-10 abs


L14  ANSWER 1 OF 28  CABA COPYRIGHT 2001 CABI
AB   Previous studies have presented indirect evidence that the transposase of
     the maize transposable element Activator (TPase) is active as an oligomer
     and forms inactive macromolecular complexes expressed in large amounts.
     Here, a dimerization domain at the C terminus of the protein is identified
     and characterized. This domain is the most highly conserved region in the
     transposases of elements belonging to the Activator superfamily (hAT
     element superfamily) and contains a characteristic signature motif. The
     isolated dimerization domain forms extremely stable dimers in vitro.
     Interestingly, mutations in 5 of the 6 conserved residues of the signature
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motif do not affect in vitro dimerization, whereas mutations in other, less strictly conserved residues of the signature motif do. Loss of dimerization in vitro correlates with loss of TPase activity in vivo. As revealed by in situ immunofluorescence staining of mutant TPase proteins, the dimerization domain also is involved in forming inactive macromolecular aggregates when overexpressed, and the TPase contains one or more additional interaction functions.

L14 ANSWER 2 OF 28 CABA COPYRIGHT 2001 CABI
AB The mobility of maize transposable element Activator (Ac) is dependent on the 11-bp terminal inverted repeats (IRs) and approximately 250 subterminal nucleotides at each end. These sequences flank the coding region for the transposase (TPase) protein, which is required for the transposition reaction. Here, it is shown that Ac TPase has a bipartite DNA binding domain, and recognizes the IRs and subterminal sequences in the Ac ends. TPase binds cooperatively to repetitive ACG and TCG sequences, of which 25 copies are found in the 5' and 20 copies in the 3' subterminal regions. TPase affinity is highest when these sites are flanked on the 3' side by an additional G residue (A/TCGG), which is found at 75% of binding sites. Moreover, TPase binds specifically to the Ac IRs, albeit with much lower affinity. Two mutations within the IRs that immobilize Ac abolish TPase binding completely. The basic DNA binding domain of TPase is split into two subdomains. Binding to the subterminal motifs is accomplished by the C-terminal subdomain alone, whereas recognition of the IRs requires the N-terminal subdomain in addition. Furthermore, TPase is extremely flexible in DNA binding. Two direct or inverted binding sites are bound equally well, and sites that are five to twelve bases apart are similarly well bound. The consequences of these findings for the Ac transposition reaction are discussed.

L14 ANSWER 3 OF 28 CABA COPYRIGHT 2001 CABI
AB The maize transposable element Activator (Ac) transposes after replication from only one of the two daughter chromatids. It has been suggested that DNA methylation in conjunction with methylation-sensitive transposase binding to DNA may control the association of Ac transposition and replication. This paper presents a detailed genomic sequencing analysis of the cytosine methylation patterns of the transposase binding sites within both Ac ends in the wx-m9::Ac allele, where Ac is inserted into the tenth exon of the Waxy gene. The Ac elements in wx-m9::Ac kernels exhibit intriguing methylation patterns and fall into two distinct groups. Approximately 50% of the elements are fully unmethylated at cytosine residues through the 256 nucleotides at the 5' end (the promoter end). The other elements are partially methylated between Ac residues 27 and 92. In contrast, at the 3' end, all Ac molecules are heavily methylated between residues 4372 and 4554. The more internally located Ac sequences and the flanking Waxy DNA are unmethylated. Although most methylated cytosines in Ac are in the symmetrical CpG and CpNpG arrangements, non-symmetrical cytosine methylation is also common in the hypermethylated regions of Ac. These results suggest a model in which differential activation of transposon ends by hemimethylation controls the chromatid selectivity of transposition and the association with replication.

L14 ANSWER 4 OF 28 CABA COPYRIGHT 2001 CABI
AB A fusion of the strong cauliflower mosaic virus 35S promoter to the Activator (Ac) transposase (TPASE) gene did not trigger excision of Dissociation (Ds) continuously during tobacco cotyledon development, although once activated, the 35S promoter remains active throughout embryogeny. Epistasis studies where 35S:TPASE is in trans with later-acting fusions indicated that transient effectiveness for excision results from this fusion inhibiting its own action and that of other TPASE

sources. Inhibition depends on the strength of TPASE expression, since fusions of the [redacted] promoter to a TPASE cDNA accumulated 30-fold lower amounts of TPASE mRNA than the 35S:TPASE gene fusion and did not inhibit excision. The role of TPASE levels in the relationship between Ac activity and Ac dosage in maize is discussed.

L14 ANSWER 5 OF 28 CABA COPYRIGHT 2001 CABI
AB The open reading frame coding for the transposase gene of the maize transposon Activator (Ac) was expressed in transgenic tobacco plants under the control of the promoter of the inducible gene for pathogenesis-related protein 1a (PR-1a). Excision of a non-autonomous transposable element (Ds) from chimaeric beta -glucuronidase (GUS) and luciferase reporter gene constructs was employed to analyse the induction of the Ac transposase by external and by internal stimuli. Applying the GUS histochemical assay, Ds excision events were detected in leaves, stems and roots after treatment of regenerating shoots with salicylic acid (SA). Varying the SA induction procedure led to different Ds excision patterns in leaves and in roots. Furthermore, Ds excision events were also observed in non-treated, older transgenic plants in the green leaves, but not in germinal cells. Thus, the PR-1a promoter/Ac transposase gene fusion, together with the improved methods for induction of this chimaeric gene, may provide a valuable tool for studying basic mechanisms of Ac transposition and for developing modified transposable element systems suitable for gene tagging in higher plants.

L14 ANSWER 6 OF 28 CABA COPYRIGHT 2001 CABI
AB The Activator (Ac) element of maize is active at a low frequency in Arabidopsis thaliana. To determine whether this is due to poor expression of the Ac transposase gene, 19 Arabidopsis transformants containing fusions of the octopine synthase (ocs), nopaline synthase (nos), cauliflower mosaic caulimovirus (CaMV) 35S, or Ac promoters to the transposase open reading frame were studied. These transformants were examined both for their ability to drive excision of a Dissociation (Ds) element from a streptomycin resistance gene and for the abundance of the transposase mRNA. Most transformants containing the CaMV 35S fusion have high levels of transposase transcript and drive high frequencies of somatic and germinal excision. These results demonstrated that Arabidopsis contains all of the host functions required for high frequency excision of Ds. Moreover, transposase mRNA abundance varied about 1000-fold among transformants; this variation made it possible to demonstrate that for the Ac, ocs, and CaMV 35S fusions, raising the mRNA level is closely correlated with increasing excision frequency. Data is discussed in relation to the behaviour of Ac in Arabidopsis, maize and tobacco.

L14 ANSWER 7 OF 28 CABA COPYRIGHT 2001 CABI
AB To explore the effects of altering the level of Activator (Ac) transposase (TPase) expression, a series of plasmids was constructed in which heterologous promoters were fused to the TPase gene. Promoters for the cauliflower mosaic caulimovirus (CaMV) 35S transcript and the octopine synthase (ocs) and nopaline synthase (nos) genes were tested. These fusions, and constructs expressing TPase from the wild-type Ac promoter, were introduced into tobacco and their activity was monitored by crossing to a line carrying Dissociation (Ds) in a streptomycin phosphotransferase gene (Ds::SPT). The SPT marker provides a record of somatic excisions of Ds that occur during embryo development. The patterns of somatic variegation that resulted from transactivation by each fusion were

distinct and st[redacted]ingly different from the patte[redacted]triggered by the wild-type Ac co[redacted]ructs. Unlike wild-type Ac, wh[redacted] caused transposition throughout embryo development, each fusion gave rise to sectors of discrete size. Sectors triggered by the CaMV 35S fusion were largest, ocs sectors were intermediate, and nos were smallest. These patterns appear to indicate differential timing of the activation of these promoters during embryogeny. Measurement of transcript abundance for each transformant indicated that the CaMV 35S-transformed plants accumulated approximately 1000-fold more TPase mRNA than plants containing wild-type Ac, whereas ocs and nos-transformed lines accumulated about 100-fold and 20-fold higher levels, respectively. Measurements of germinal excision frequencies driven by the chimaeric TPase fusions, however, indicated that increasing transcription does not necessarily result in an increase in germinal excision. These measurements showed that the ocs and nos fusions have very low rates of germinal excision. Only the CaMV 35s fusion transformants were found to have higher rates than the Ac constructs, although significant pod-to-pod variation was observed. Gel blot analysis of DNA from progeny carrying germinal excision events resulting from the CaMV 35S fusions showed that excision is associated with reinsertion and that siblings sometimes carry the same transposition events. These findings suggest that in tobacco there is no direct proportionality between TPase expression and Ac-Ds transposition activity. This possibility has important implications for understanding the regulation of Ac transposition and for designing efficient gene tagging systems.

L14 ANSWER 8 OF 28 CAPLUS COPYRIGHT 2001 ACS

AB Previous studies have presented indirect evidence that the transposase of the maize transposable element Activator (Ac TPase) is active as an oligomer and forms inactive macromol. complexes expressed in large amts. Here, we have identified and characterized a dimerization domain at the C terminus of the protein. This domain is the most highly conserved region in the transposases of elements belonging to the Activator superfamily (hAT element superfamily) and contains a characteristic signature motif. The isolated dimerization domain forms extremely stable dimers in vitro. Interestingly, mutations in five of the six conserved residues of the signature motif do not affect in vitro dimerization, whereas mutations in other, less strictly conserved residues of the signature motif do. Loss of dimerization in vitro correlates with loss of TPase activity in vivo. As revealed by in situ immunofluorescence staining of mutant TPase proteins, the dimerization domain also is involved in forming inactive macromol. aggregates when overexpressed, and the TPase contains one or more addnl. interaction functions.

L14 ANSWER 9 OF 28 CAPLUS COPYRIGHT 2001 ACS

AB In plants, the frequency of spontaneous intrachromosomal homologous recombination is low. Here, we show that a maize transposable element greatly stimulates intrachromosomal homologous recombination between direct repeat sequences in Arabidopsis. Plants were transformed with a construct (GU-Ds-US) contg. a Ds (Dissocn.) transposable element inserted between two partially deleted GUS reporter gene segments. Homologous recombination between the overlapping GUS fragments generates clonal sectors visible upon staining for GUS activity. Plants contg. the GU-Ds-US construct and a source of Ac (**Activator**) **transposase** showed an over 1000-fold increase in the incidence of recombination relative to plants contg. the same construct but lacking transposase. Transposon-induced recombination was obsd. in vegetative and floral organs, and several germinally transmitted events were recovered. Transposon-induced recombination appears to be a general phenomenon in plants, and thus may have contributed to genome evolution by inducing

deletions between repeated sequences.

L14 ANSWER 10 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB The mobility of maize transposable element Activator (Ac) is dependent on the 11-bp terminal inverted repeats (IRs) and approx. 250 subterminal nucleotides at each end. These sequences flank the coding region for the transposase (TPase) protein, which is required for the transposition reaction. Here we show that Ac TPase has a bipartite DNA binding domain, and recognizes the IRs and subterminal sequences in the Ac ends. TPase binds cooperatively to repetitive ACG and TCG sequences, of which 25 copies are found in the 5' and 20 copies in the 3'-subterminal regions. TPase affinity is highest when these sites are flanked on the 3' side by an addnl. G residue (A/TCGG), which is found at 75% of binding sites. Moreover, TPase binds specifically to the Ac IRs, albeit with much lower affinity. Two mutations within the IRs that immobilize Ac abolish TPase binding completely. The base DNA binding domain of TPase is split into two subdomains. Binding to the subterminal motifs is accomplished by the C-terminal subdomain alone, whereas recognition of the IRs requires the N-terminal subdomain in addn. Furthermore, TPase is extremely flexible in DNA binding. Two direct or inverted binding sites are found equally well, and sites that are five to twelve bases apart are similarly well bound. The consequences of these findings for the Ac transposition reaction are discussed.

=> d l14 11-20 abs

L14 ANSWER 11 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB The maize transposable element Activator (Ac) transposes after replication from only one of the two daughter chromatids. It has been suggested that DNA methylation in conjunction with methylation-sensitive transposase binding to DNA may control the assocn. of Ac transposition and replication. We present here a detailed genomic sequencing anal. of the cytosine methylation patterns of the transposase binding sites within both Ac ends in the wx-m9::Ac allele, where Ac is inserted into the tenth exon of the Waxy gene. The Ac elements in wx-m9::Ac kernels exhibit intriguing methylation patterns and fall into two distinct groups. Approx. 50% of the elements are fully unmethylated at cytosine residues through the 256 nucleotides at the 5' end (the promoter end). The other half is partially methylated between Ac residues 27 and 92. In contrast, at the 3' end, all Ac mols. are heavily methylated between residues 4372 and 4554. The more internally located Ac sequences and the flanking Waxy DNA are unmethylated. Although most methylated cytosines in Ac are in the sym. CpG and CpNpG arrangements, nonsym. cytosine methylation is also common in the hypermethylated regions of Ac. These results suggest a model in which differential activation of transposon ends by hemimethylation controls the chromatid selectivity of transposition and the assocn. with replication.

L14 ANSWER 12 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB Properties of two deletion derivs. of the transposase protein (TPase) of maize transposable element Ac were examd. in transgenic tobacco. The wild-type and mutant TPases were expressed as fusions to the cauliflower mosaic virus 35S promoter. A deletion of 102 amino acids from the N-terminus, TPase(103-807), induces Ds excisions from a SPT::Ds reporter

locus with a hi[redacted]r frequency than the wild-type [redacted]ase. The increased transpositional [redacted]tivity of TPase(103-807) is a [redacted]inant trait, as seedlings coexpressing truncated and wild-type TPase show the characteristic TPase(103-807) variegation phenotype. A transpositionally inactive TPase deletion deriv. lacking 188 amino acids from the N-terminus inhibits the transpositional activity of the wild-type TPase.

L14 ANSWER 13 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB The nuclear localization sequences (NLSs) of the Ac transposase (TPase) protein have been characterized by indirect immunofluorescence detection of TPase deletion derivs. and TPase/.beta.-glucuronidase (GUS) fusion proteins in transiently transfected Petunia cells. The TPase contains three NLSs near its amino-terminal end, NLS(44-62), NLS(159-178) and NLS(174-206), each of which is sufficient to redirect GUS to the nucleus. Deletion of the N-terminal 102 TPase residues including NLS(44-62) results in strongly reduced nuclear import of the truncated TPase. NLS(44-62) and NLS(159-178) are bipartite NLSs, whereas the structure of NLS(174-206) does not allow a classification into one of the three major NLS categories. NLS(174-206) overlaps with the basic DNA-binding domain of TPase. A substitution of two amino acids in this segment (His191.fwdarw.Arg and Arg193.fwdarw.His) results in a total loss of DNA-binding activity, but retains reduced NLS activity. Accordingly, the two functions can be sepd. In addn., the authors show that a NLS-deficient 71 kDa TPase deriv. is co-imported into the nucleus in the presence of wild-type TPase.

L14 ANSWER 14 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB The open reading frame coding for the transposase gene of the maize transposon Activator (Ac) was expressed in transgenic tobacco plants under the control of the promoter of the inducible gene for pathogenesis-related protein 1a (PR-1a). Excision of a non-autonomous transposable element (Ds) from chimeric .beta.-glucuronidase (GUS) and luciferase reporter gene constructs was employed to analyze the induction of the Ac transposase by external and by internal stimuli. Applying the GUS histochem. assay, Ds excision events were detected in leaves, stems, and roots after treatment of regenerating shoots with salicylic acid (SA). Varying the SA induction procedure led to different Ds excision patterns in leaves and in roots. Furthermore, Ds excision events were also obsd. in non-treated, older transgenic plants in the green leaves, but not in germinal cells. Thus, the PR-1a promoter/Ac transposase gene fusion, together with the improved methods for induction of this chimeric gene, may provide a valuable tool for studying basic mechanisms of Ac transposition and for developing modified transposable element systems suitable for gene tagging in higher plants.

L14 ANSWER 15 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB The transposase (TPase) of the maize transposon Activator (Ac) accumulates in the nuclei of maize endosperm and transfected Petunia protoplasts, where it aggregates into rod-like structures about 2 .mu.m in length. In petunia protoplasts the amt. of TPase aggregates increases with the strength of the promoter fused to the Ac-coding region. The excision frequency of a Ds element, however, does not increase proportionally. The data suggest that the aggregated TPase is not responsible for the mobilization of the Ds element, but rather is a transpositionally inactive form of the protein. In contrast to the full-length TPase, a functional,

N-terminally truncated TPase deriv. is inefficiently transported into the nucleus at high expression levels and aggregates predominantly in the cytoplasm. Accordingly, the N-terminus of the TPase is involved in nuclear localization and/or aggregation.

L14 ANSWER 16 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB A fusion of the strong cauliflower mosaic virus 35S promoter to the Activator (Ac) transposase (TPASE) gene does not trigger excision of Dissocn. (Ds) continuously during tobacco cotyledon development, although once activated, the 35S promoter remains active throughout embryogeny. Epistasis studies where 35S:TPASE is in trans with later-acting fusions indicate that transient effectiveness for excision results from this fusion inhibiting its own action and that of other TPASE sources. Inhibition depends on the strength of TPASE expression, since fusions of the 35S promoter to a TPASE cDNA accumulate 30-fold lower amts. of TPASE mRNA than the 35S:TPASE gene fusion and do not inhibit excision. The authors discuss the role of TPASE levels in the curious relationship between Ac activity and Ac dosage in maize.

L14 ANSWER 17 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB To explore the effects of altering the level of Activator (Ac) transposase (TPase) expression, a series of plasmids was constructed in which heterologous promoters were fused to the TPase gene. Promoters for the cauliflower mosaic virus (CaMV) 35S transcript and the octopine synthase (ocs) and nopaline synthase (nos) genes were tested. These fusions, and constructs expressing TPase from the wild-type Ac promoter, were introduced into tobacco, and their activity was monitored by crossing to a line carrying Dissocn. (Ds) in a streptomycin phosphotransferase gene (Ds::SPT). The SPT marker provides a record of somatic excisions of Ds that occur during embryo development. The patterns of somatic variegation that resulted from transactivation by each fusion were distinct and strikingly different from the pattern triggered by the wild-type Ac, which caused transposition throughout embryo development, each fusion gave rise to sectors of discrete size. Sectors triggered by the CaMV 35S fusion were largest, ocs sectors were intermediate, and nos were smallest. These patterns appear to indicate differential timing of the activation of these promoters during embryogeny. Measurement of transcript abundance for each transformant indicated that the CaMV 35S-transformed plants accumulated .apprx.1000-fold more TPase mRNA than do plants contg. wild-type Ac, whereas ocs- and nos-transformed lines accumulated about 100-fold and 20-fold higher levels, resp. Measurements of germinal excision frequencies driven by the chimeric TPase fusions, however, indicated that increasing transcription does not necessarily result in an increase in germinal excision. These measurements showed that the ocs and nos fusions have very low rates of germinal excision. Only the CaMV 35S fusion transformants were found to have higher rates than the Ac constructs, although significant pod-to-pod variation was obsd. Gel blot anal. of DNA from progeny carrying germinal excision events resulting from the CaMV 35S fusion showed that excision is assocd. with reinsertion and that siblings sometimes carry the same transposition events. These findings suggest that in tobacco there is no direct proportionality between TPase expression and Ac-Ds transposition activity. This possibility has important implications for understanding the regulation of Ac transposition and for designing efficient gene tagging systems.

L14 ANSWER 18 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB The Activator (Ac) element of maize is active at low frequency in Arabidopsis. To det. whether this is due to poor expression of the Ac transposase gene, 19 Arabidopsis transformants were studied contg. fusions of the octopine synthase (ocs), nopaline synthase (nos), cauliflower mosaic virus (CaMV) 35S, or Ac promoters to the transposase open reading frame. These transformants were examd. both for their ability to drive excision of a Dissocn. (Ds) element from a streptomycin resistance gene and for the abundance of the transposase mRNA. Most transformants contg. the CaMV 35S fusion have high levels of transposase transcript and drive high frequencies of somatic and germinal excision. Thus, Arabidopsis contains all of the host functions required for high frequency excision of Ds. Moreover, transposase mRNA abundance varied about 1000-fold among our transformants; this variation showed that for the Ac, ocs, and CaMV 35S fusions, raising the mRNA level is closely correlated with increasing excision frequency. The data were discussed in relation to the behavior of Ac in Arabidopsis, maize, and tobacco.

L14 ANSWER 19 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
AB In plants, the frequency of spontaneous intrachromosomal homologous recombination is low. Here, we show that a maize transposable element greatly stimulates intrachromosomal homologous recombination between direct repeat sequences in Arabidopsis. Plants were transformed with a construct (GU-Ds-US) containing a Ds (Dissociation) transposable element inserted between two partially deleted GUS reporter gene segments. Homologous recombination between the overlapping GUS fragments generates clonal sectors visible upon staining for GUS activity. Plants containing the GU-Ds-US construct and a source of Ac (**Activator**) **transposase** showed an over 1000-fold increase in the incidence of recombination relative to plants containing the same construct but lacking transposase. Transposon-induced recombination was observed in vegetative and floral organs, and several germinally transmitted events were recovered. Transposon-induced recombination appears to be a general phenomenon in plants, and thus may have contributed to genome evolution by inducing deletions between repeated sequences.

L14 ANSWER 20 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
AB An inducible transposon tagging technique in plants with a large genome was accessed. The open reading frame coding for the transposase gene of the maize transposon Activator (Ac) was expressed in transgenic tobacco plants under the control of the promoter of the inducible gene for pathogenesis-related protein 1a (PR-1a). Based on the native PR-1a promoter, several inducible promoters were constructed in order to induce variability of the Ac transposase expression level. Excision of a non-autonomous transposable element (Ds) from the chimeric beta-glucuronidase gene construct was employed to analyze the induction of the Ac transposase by salicylic acid (SA). Applying the beta-glucuronidase histochemical assay, Ds excision efficiency was determined in the regeneration calli after treatment of a tobacco leaf disc with SA. A 111-bp regulatory element, located between nucleotides - 699 to - 588 of the PR-1a promoter, was fused to the 89-bp CaMV35S core promoter to serve as the inducible promoter, PRAD. When the PRDELTAD promoter was fused with the Ac transposase gene and induced by SA, it triggered a 26-fold higher Ds excision efficiency than the native PR-1a promoter fusion. Furthermore, Ds excision events occurred mainly in the regeneration calli at day 8 after induction with SA. Because Ds excision events could be induced in

such a short period, an alternative gene tagging strategy is suggested.

=> d l14 21-28 abs

L14 ANSWER 21 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
AB The mobility of maize transposable element Activator (Ac) is dependent on the 11-bp terminal inverted repeats (IRs) and approximately 250 subterminal nucleotides at each end. These sequences flank the coding region for the transposase (TPase) protein, which is required for the transposition reaction. Here we show that Ac TPase has a bipartite DNA binding domain, and recognizes the IRs and subterminal sequences in the Ac ends. TPase binds cooperatively to repetitive ACG and TCG sequences, of which 25 copies are found in the 5' and 20 copies in the 3' subterminal regions. TPase affinity is highest when these sites are flanked on the 3' side by an additional G residue (A/TCGG), which is found at 75% of binding sites. Moreover, TPase binds specifically to the Ac IRs, albeit with much lower affinity. Two mutations within the IRs that immobilize Ac abolish TPase binding completely. The basic DNA binding domain of TPase is split into two subdomains. Binding to the subterminal motifs is accomplished by the C-terminal subdomain alone, whereas recognition of the IRs requires the N-terminal subdomain in addition. Furthermore, TPase is extremely flexible in DNA binding. Two direct or inverted binding sites are bound equally well, and sites that are five to twelve bases apart are similarly well bound. The consequences of these findings for the Ac transposition reaction are discussed.

L14 ANSWER 22 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
AB The maize transposable element Activator (Ac) transposes after replication from only one of the two daughter chromatids. It has been suggested that DNA methylation in conjunction with methylation-sensitive transposase binding to DNA may control the association of Ac transposition and replication. We present here a detailed genomic sequencing analysis of the cytosine methylation patterns of the transposase binding sites within both Ac ends in the wx-m9::Ac allele, where Ac is inserted into the tenth axon of the Waxy gone. The Ac elements in wx-m9::Ac kernels exhibit intriguing methylation patterns and fall into two distinct groups. Approximately 50% of the elements are fully unmethylated at cytosine residues through the 256 nucleotides at the 5' end (the promoter end). The other half is partially methylated between Ac residues 27 and 92. In contrast, at the 3' end, all Ac molecules are heavily methylated between residues 4372 and 4554. The more internally located Ac sequences and the flanking Waxy DNA are unmethylated. Although most methylated cytosines in Ac are in the symmetrical CpG and CpNpG arrangements, nonsymmetrical cytosine methylation is also common in the hypermethylated regions of Ac. These results suggest a model in which differential activation of transposon ends by hemimethylation controls the chromatid selectivity of transposition and the association with replication.

L14 ANSWER 23 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
AB The open reading frame coding for the transposase gene of the maize transposon Activator (Ac) was expressed in transgenic tobacco plants under the control of the promoter of the inducible gene for pathogenesis-related protein 1a (PR-1a). Excision of a non-autonomous transposable element (Ds) from chimeric beta-glucuronidase (GUS) and luciferase reporter gene

constructs was employed to analyze the induction of the Ac transposase by external and by internal stimuli. Applying the GUS histochemical assay, Ds excision events were detected in leaves, stems, and roots after treatment of regenerating shoots with salicylic acid (SA). Varying the SA induction procedure led to different Ds excision patterns in leaves and in roots. Furthermore, Ds excision events were also observed in non-treated, older transgenic plants in the green leaves, but not in germinal cells. Thus, the PR-1a promoter/Ac transposase gene fusion, together with the improved methods for induction of this chimeric gene, may provide a valuable tool for studying basic mechanisms of Ac transposition and for developing modified transposable element systems suitable for gene tagging in higher plants.

L14 ANSWER 24 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
AB We have recently shown that a plasmid-borne Dissociation (Ds) element can excise from extrachromosomal plasmid DNA and integrate into a plant genome in the presence of the Activator (Ac) transposase. Ds and Ac-carrying plasmids were used to co-transform Nicotiana plumbaginifolia protoplasts. Transgenic plants were regenerated and analyzed. Here we describe further characterization of the system and discuss its efficiency in terms of DNA transformation and transposon tagging.

L14 ANSWER 25 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
AB A fusion of the strong cauliflower mosaic virus 35S promoter to the Activator (Ac) transposase (TPASE) gene does not trigger excision of Dissociation (Ds) continuously during tobacco cotyledon development, although once activated, the 35S promoter remains active throughout embryogeny. Epistasis studies where 35S:TPASE is in trans with later-acting fusions indicate that transient effectiveness for excision results from this fusion inhibiting its own action and that of other TPASE sources. Inhibition depends on the strength of TPASE expression, since fusions of the 35S promoter to a TPASE cDNA accumulate 30-fold lower amounts of TPASE mRNA than the 35S:TPASE gene fusion and do not inhibit excision. We discuss the role of TPASE levels in the curious relationship between Ac activity and Ac dosage in maize.

L14 ANSWER 26 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
AB Overexpression of the Activator (Ac) transposase gene in Arabidopsis thaliana resulted in a minimal germinal transposition frequency of 27% in which independent Dissociation (Ds) transposition event were observed. Molecular analysis of 45 F1 generations Ac/Ds plants indicated that high rates of somatic excision had occurred, and independent germinal insertions were identified in F2 generation progeny plants. A tandem cauliflower mosaic virus (CaMV) promoter fused to two different Ac coding sequences significantly increased the rate of Ds transposition. The CaMV-Ac fusions activated single and multiple copies of two different Ds elements, DsDHFR and Ds35S-1, and reciprocal crosses resulted in similar transposition frequencies. The improved rate of independent germinal transposition observed makes Arabidopsis an ideal system for insertional mutagenesis.

L14 ANSWER 27 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
AB The Activator (Ac) element of maize is active at a low frequency in Arabidopsis. To determine whether this is due to poor expression of the Ac transposase gene, we obtained and studied 19 Arabidopsis transformants containing fusions of the octopine synthase (ocs), nopaline synthase (nos), cauliflower mosaic virus (CaMV) 35S, or Ac promoters to the transposase open reading frame. These transformants were examined both for their ability to drive excision of a Dissociation (Ds) element from a streptomycin resistance gene and for the abundance of the transposase

mRNA. Most transformants containing the CaMV 35S fusion have high levels of transposase transcript and drive high frequencies of somatic and germinal excision. These results demonstrated that Arabidopsis contains all of the host functions required for high frequency excision of Ds. Moreover, transposase mRNA abundance varied about 1000-fold among our transformants; this variation enabled us to demonstrate that for the Ac, ocs, and CaMV 35S fusions, raising the mRNA level is closely correlated with increasing excision frequency. We discuss our data in relation to the behavior of Ac in Arabidopsis, maize, and tobacco.

L14 ANSWER 28 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
AB To explore the effects of altering the level of Activator (Ac) transposase (TPase) expression, a series of plasmids was constructed in which heterologous promoters were fused to the TPase gene. Promoters for the cauliflower mosaic virus (CaMV) 35S transcript and the octopine synthase (ocs) and nopaline synthase (nos) genes were tested. These fusions, and constructs expressing TPase from the wild-type Ac promoter, were introduced into tobacco, and their activity was monitored by crossing to a line carrying Dissociation (Ds) in a streptomycin phosphotransferfase gene (Ds::SPT). The SPT marker provides a record of somatic excisions of Ds that occur during embryo development. The patterns of somatic variegation that resulted from transactivation by each fusion were distinct and strikingly different from the pattern triggered by the wild-type Ac constructs. Unlike wild-type Ac, which caused transposition throughout embryo development, each fusion gave rise to sectors of discrete size. Sectors triggered by the CAM 35S fusion were largest, ocs sectors were intermediate, and nos were smallest. These patterns appear to indicate differential timing of the activation of these promoters during embryogeny. Measurement of transcript abundance for each transformant indicated that the CaMV 35S-transformed plants accumulated approximately 1000-fold more TPase mRNA than plants containing wild-type Ac, whereas ocs- and nos-transformed lines accumulated about 100-fold and 10-fold higher levels, respectively. Measurements of germinal excision frequencies driven by the chimeric TPase fusions, however, indicated that increasing transcription does not necessarily result in an increase in germinal excision. These measurements showed that the ocs and nos fusions have very low rates of gereminal excision. Only the CaMV 35S fusion transformants were found to have higher rates than the Ac constructs, although significant pod-to-pod variation was observed. Gel blot analysis of DNA from progeny carrying germinal excision events resulting from the CaMV 35S fusion showed that the excision is associated with reinsertion and that siblings sometimes carry the same transposition events. These findings suggest that in tobacco there is no direct proportionality between TPase expression and Ac-Ds transposition activity. This possibility has important implications for understanding the regulation of Ac transposition and for designing efficient gene tagging systems.

=> d hist

(FILE 'HOME' ENTERED AT 15:36:56 ON 19 JAN 2001)

FILE 'CAPLUS, BIOSIS, CEABA-VTB' ENTERED AT 15:41:27 ON 19 JAN 2001
L1 944349 S PLANT

FILE 'CABA, CAPLUS, BIOSIS, CEABA-VTB' ENTERED AT 15:43:54 ON 19 JAN 2001
L2 1742997 S PLANT

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L3          5468 S CYTOTOXIN
L4           104 S L2 AND L3
L5          3383 S RECOMBINASE
L6             0 S L4 AND L5
L7           124 S L2 AND L5
L8            24 S L7(P)NUCLEOTIDE#
L9            21 S L7(P)NUCLEOTIDE
L10           97 S L2(P)L5
L11       134909 S ZEA(W)MAYS
L12        54532 S L2 AND L11
L13            2 S L12 AND L5
L14           28 S ACTIVATOR(W)TRANSPOSASE
L15            0 S L14(P)L11
L16            0 S L11(P)L14

=> d l13 1-2 abs


L13  ANSWER 1 OF 2  CABA COPYRIGHT 2001 CABI
AB   With a view to developing more efficient DNA transformation procedures,
     yeast FLP/FRT site-specific recombination system has been shown to
     function in maize and rice protoplasts. FLP recombinase activity
     was monitored by reactivation of beta -glucuronidase (GUS) expression
from
     vectors containing the gusA gene inactivated by insertion of two FRTs
(FLP
     recombination targets) and a 1.31 kb DNA fragment. The stimulation of GUS
     activity in protoplasts co-transformed with vectors containing
     FTR-inactivated gusA gene and a chimaeric FLP gene depended on both the
     expression of the FLP recombinase and the presence and structure
     of the FRT sites. The FLP enzyme could mediate inter- and intramolecular
     recombination in plant protoplasts. These results provide
     evidence that a yeast recombination system can function efficiently in
     plant cells, and that its performance can be manipulated by
     structural modification of the FRT sites.

L13  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2001 ACS
AB   Nucleic acid sequences encoding two RAD51 recombinases active in maize
     plants are provided.  Full-length cDNA sequences from a maize tassel cDNA
     library,including the ZmRAD51 coding sequences and unique 3-untranslated
     regions which are useful as RFLP probes, are also provided.  RFLP mapping
     indicates that the Zea mays genome contains 2 genes
     encoding different variants of the ZmRAD51 recombinase enzyme,
     one mapped to chromosome 7 and the other on chromosome 3.  The prodn. of
     plasmids contg. a nucleic acid sequence encoding a ZmRAD51 fusion
protein,
     as well as the use of the plasmids to introduce the ZmRAD51 coding
     sequence into a host cell, such as maize cell, are also disclosed.


=> d his


     (FILE 'HOME' ENTERED AT 15:36:56 ON 19 JAN 2001)

     FILE 'CAPLUS, BIOSIS, CEABA-VTB' ENTERED AT 15:41:27 ON 19 JAN 2001
L1        944349 S PLANT

     FILE 'CABA, CAPLUS, BIOSIS, CEABA-VTB' ENTERED AT 15:43:54 ON 19 JAN 2001
L2       1742997 S PLANT
L3          5468 S CYTOTOXIN
L4           104 S L2 AND L3
L5          3383 S RECOMBINASE
L6             0 S L4 AND L5
L7           124 S L2 AND L5
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L8          24 S L7 (3)NUCLEOTIDE#
L9          21 S L7 (2)NUCLEOTIDE
L10         97 S L2(P)L5
L11     134909 S ZEA(W)MAYS
L12      54532 S L2 AND L11
L13          2 S L12 AND L5
L14         28 S ACTIVATOR(W)TRANSPOSASE
L15          0 S L14(P)L11
L16          0 S L11(P)L14

=> d l8 1-10 abs
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L8 ANSWER 1 OF 24 CABA COPYRIGHT 2001 CABI
AB The **recombinase** activating genes (RAG1 and RAG2; endonucleases involved in V(D)J recombination) of Japanese pufferfish were isolated, together with a possible homologue of the **plant** 1-aminocyclopropane-carboxylate synthase (ACS) gene, and sequenced. The structure of the RAG genes was identical to that of their homologues from other fish species but the intergenic region was smaller. Reverse transcription-PCR detected RAG transcripts in the kidneys of adult pufferfish.

L8 ANSWER 2 OF 24 CABA COPYRIGHT 2001 CABI
AB A cDNA (recA-AT) from Arabidopsis thaliana encoding a chloroplast-located homologue of bacterial RecA protein, was introduced into a plasmid appropriate for in vitro transcription and translation. Translation with 35S-labelled methionine demonstrated the uptake of the protein into isolated pea (Pisum sativum) chloroplasts, and processing to a mature size. Uptake experiments using both 35S-methionine and 3H-leucine demonstrated the chloroplast-targeting function of the N-terminal 51 amino acids. The labelled protein was subject to sequential amino acid hydrolyses, and radioactivity was measured in each round. Induction of gene transcription in A. thaliana leaves infiltrated with the DNA-damaging agent, methyl methane-sulfonate, was shown by Northern blot analysis. Further constructs were made for overexpression of the gene in Escherichia coli and extracts were shown to have RecA activity using an assay for DNA strand transfer. The protein was purified to close to homogeneity using methods developed for E. coli RecA isolation. **Nucleotide** sequence data reported here have been submitted to the EMBL/GenBank/DDBJ database under accession number M93899 for the cDNA and L15229 for the genomic sequence.

L8 ANSWER 3 OF 24 CABA COPYRIGHT 2001 CABI
AB A general method of gene transfer that does not leave behind a selectable marker in the host genome is described. A luciferase (luc) gene was introduced into the tobacco genome by using the hygromycin phosphotransferase gene (hpt) as a linked selectable marker. Flanked by recombination sites from the bacteriophage P1 Cre/lox recombination system, the hpt gene was subsequently excised from the **plant** genome by the Cre **recombinase**. The Cre-catalysed excision event in the **plant** genome was precise and conservative; without loss or alteration of **nucleotides** in the recombinant site. After removal of the Cre-encoding locus by genetic segregation, plants were obtained that had incorporated only the desired transgene. Gene transfer without the incorporation of antibiotic-resistance markers in the host genome should ease public concerns over the field release of transgenic organisms expressing such traits. Moreover, it would obviate the need for different selectable markers in subsequent rounds of gene transfer into the same host.

L8 ANSWER 4 OF 24 CAPLUS COPYRIGHT 2001 ACS

AB This invention relates to cDNAs encoding corn and wheat gene RAD51 recombinases. The invention also relates to the construction of a chimeric gene encoding all or a substantial portion of the recombination protein, in sense or antisense orientation, wherein expression of the chimeric gene results in prodn. of altered levels of the recombination protein in a transformed host cell. Also disclosed is use of the chimeric gene for pos. selection of transformed **plant** cells.

L8 ANSWER 5 OF 24 CAPLUS COPYRIGHT 2001 ACS
AB The present invention is directed to isolated promoter sequences from seed-specific genes, such as KNAT411 . When operably linked to either the coding sequence of a heterologous gene or a sequence complementary to a native **plant** gene, the subject promoters direct expression of the coding sequence or complementary sequence in a **plant** seed, including the early embryo. The promoter sequences are useful in expression cassettes and expression vectors for the transformation of plants. Also provided are methods of directing seed-specific expression of a gene or sequence complementary to a native **plant** gene by introducing into a **plant** cell an isolated nucleic acid comprising a subject promoter operably linked to said gene or complementary sequence. Methods for activating a site-specific recombination system in the early embryo of a seed by transforming a **plant** with an expression cassette comprising a subject promoter operably linked to a **recombinase** gene are also provided. Thus, the A. thaliana KNAT411 gene promoter was cloned and sequenced. This gene was found to be active very early in embryogenesis, much earlier than other known seed-specific promoters. Southern anal. indicated that there was only one KNAT411 gene, but there were several KNAT411-like sequences in the A. thaliana genome. The KNAT411 gene was detd. to have five exons sepd. by four introns. The obsd. position of the third intron (inside the ELK domain) and of the fourth intron (interrupting the homeodomain) is characteristic of knotted genes.

L8 ANSWER 6 OF 24 CAPLUS COPYRIGHT 2001 ACS
AB The invention relates to methods for inhibiting, cloning, modifying, or labeling an endogenous DNA sequence using compns. comprising recombinases in combination with exogenous polynucleotides contg. "anchoring" or "locking" sequences. Preferred recombinases include Escherichia coli RecA protein, and eukaryotic Rad51 **recombinase**. The anchoring sequences serve to stabilize structures formed by the exogenous polynucleotides and the endogenous DNA via the formation of, for example, triplex or quadruplex structures. Double D-loops form by probes with heterologous inserts demonstrate increased kinetic stabilities compared with double D-lops formed by completely homologous probes. The stabilized structure thus can either serve to regulate gene transcription or replication, or can allow the endogenous sequences to be labeled or pulled out, i.e. cloned, or modified.

L8 ANSWER 7 OF 24 CAPLUS COPYRIGHT 2001 ACS
AB A method for introducing a site-specific mutation into a target polynucleotide sequence is presented. The method involves the use of an oligonucleotide capable of binding to the target sequence, either by triplex formation (mediated by Hoogsteen, reverse Hoogsteen or equiv. base pairing) or by Watson/Crick base pairing (in the presence of a **recombinase** enzyme). The oligonucleotide of the invention is modified by the covalent attachment of one or more electrophilic groups. When a modified oligonucleotide is bound to its target sequence, the

electrophilic group is able to interact with a nearby **nucleotide** in the target sequence, causing a modification to the **nucleotide** that results in a change in **nucleotide** sequence. Compns. used in the practice of the method are also disclosed.

L8 ANSWER 8 OF 24 CAPLUS COPYRIGHT 2001 ACS

AB Disclosed are variants of Cre **recombinase** that have broadened specificity for the site of recombination. Specifically, the disclosed variants mediate recombination between sequences other than the loxP sequence and other lox site sequences on which wild type Cre **recombinase** is active. In general, the disclosed Cre variants mediate efficient recombination between lox sites that wild type Cre can act on (referred to as wild type lox sites), between variant lox sites not efficiently utilized by wild type Cre (referred to as variant lox sites), and between a wild type lox site and a variant lox site. Also disclosed are methods of recombining nucleic acids using the disclosed Cre variants.

For example, the disclosed Cre variants can be used in any method or technique where Cre **recombinase** (or other, similar recombinases such as FLP) can be used. In addn., the disclosed Cre variants allow different alternative recombinations to be performed since the Cre variants allow much more efficient recombination between wild type lox sites and variant lox sites. Control of such alternative recombination can be used to accomplish more sophisticated sequential recombinations to achieve results not possible with wild type Cre **recombinase**.

L8 ANSWER 9 OF 24 CAPLUS COPYRIGHT 2001 ACS

AB The invention provides methods for modulating a cellular process by contacting a cell in culture with a cell process-modifying mol. attached to a translocating polypeptide. For example, in one embodiment, a cell in culture is transfected with a target gene by contacting the cell in culture with a polynucleotide (that contains the target gene) attached to a translocating polypeptide. In another embodiment, expression of a target gene product in a cell in culture that contains a target gene under control of one or more regulatory elements is modulated by contacting the cell in culture with one or more regulatory agents attached to a translocating polypeptide. The one or more regulatory agents are translocated into the cell in culture and interact therein with the one or more regulatory elements to modulate expression of the target gene product by the cell. The translocating polypeptide is selected from VP22, Antp, Protein H, histone 1, high mobility group 17 protein (HMG17), a polylysine, oligonucleotide having LARL repeats. It could also be attached to a nuclear export signal such as HIV Rev protein or heat stable inhibitor of cAPK. They are resistant to proteolysis, capable of receptor-independent and energy-free cell-membrane penetration. Use of T7 RNA polymerase to modulate a T7 promoter or HIV Rev protein to modulate the HIV Rev response element (RRE) is described. The regulatory agent may be attached to the translocating polypeptide via a linker contg. disulfide bonds, salicylhydroxamic acid (SHA), phenylboronic acid (PBA), a SHA-NHS ester, such as biotin-streptavidin complex and E. coli single stranded DNA binding protein. They may be part of a fusion protein. A single chain antibody (sFv) may be the regulatory agent. Use of **recombinase** such as Flp recognizing frt recombination sites or Cre recognizing lox recombination sites to stably integrate the target gene into genome of a cell is claimed. The target gene may be a reporter gene or a toxic

protein gene, and contain a protein tag such as myc epitope, a fluorescent peptide, or a poly His tag. A mammalian or insect cell may be contacted with an addnl. cell, prokaryotic or eukaryotic. Vectors used for the method are also claimed.

L8 ANSWER 10 OF 24 CAPLUS COPYRIGHT 2001 ACS

AB The **recombinase** activating genes (RAG1 and RAG2) encode nuclear proteins that directly mediate the mechanism V(D)J recombination process that occurs in T- and B-lymphocytes. The expression of RAG1 and RAG2 is required for the proper development of maturing lymphocytes. To identify evolutionary conserved regulatory regions adjacent to both genes we isolated and sequenced a cosmid clone contg. 43 kb of genomic DNA of the Japanese pufferfish, Fugu rubripes. Fugu has a haploid genome of 400 Mb and contains the same no. of genes as the genome of higher vertebrates. With low abundance of repetitive DNA, the genome of the pufferfish has shown to be ideal for comparative genomics. We found three complete genes, RAG1, RAG2 and ACS (a possible homolog of the **plant** 1-aminocyclopropane-carboxylate synthase gene). There is also the 5' exon of a prohormone convertase gene, possibly PACE4. The genetic structure of both RAG1 and RAG2 is identical to that found in other fish, but the size of the intergenic region is smaller in Fugu. Expression anal. by RT-PCR shows the presence of RAG transcripts in kidney of adult Fugu. The human ACS was identified in a cosmid assigned to chromosome 11p11, which is close to the location of the RAGs (11p12). This indicates conservation of linkage between human and pufferfish.

=> d l10 ibib 2,4,7,8,11,12,13,16,22

L10 ANSWER 2 OF 97 CABA COPYRIGHT 2001 CABI

ACCESSION NUMBER: 2000:138439 CABA
DOCUMENT NUMBER: 20001615705
TITLE: FLP-mediated recombination for use in hybrid plant production
AUTHOR: Luo Hong; Lyznik, L. A.; Gidoni, D.; Hodges, T. K.; Luo, H.
CORPORATE SOURCE: Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA.
SOURCE: Plant Journal, (2000) Vol. 23, No. 3, pp. 423-430. 45 ref.
ISSN: 0960-7412
DOCUMENT TYPE: Journal
LANGUAGE: English

L10 ANSWER 4 OF 97 CABA COPYRIGHT 2001 CABI

ACCESSION NUMBER: 1999:150168 CABA
DOCUMENT NUMBER: 991611043
TITLE: pBECKS2000: a novel plasmid series for the facile creation of complex binary vectors, which incorporates "clean-gene" facilities
AUTHOR: McCormac, A. C.; Elliott, M. C.; Chen, D. F.
CORPORATE SOURCE: Norman Borlaug Institute for Plant Science Research, De Montfort University, Scraptoft, Leicester LE7 9SU, UK.
SOURCE: Molecular and General Genetics, (1999) Vol. 261, No. 2, pp. 226-235. 37 ref.
ISSN: 0026-8925
DOCUMENT TYPE: Journal

LANGUAGE: English

L10 ANSWER 7 OF 97 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 97:4897 CABA
DOCUMENT NUMBER: 961611204
TITLE: Recombinase-directed chromosome engineering in plants
AUTHOR: Ow, D. W.
CORPORATE SOURCE: Plant Gene Expression Center, USDA-ARS, University of California at Berkeley, 800 Buchanan Street, Albany, California 94710, USA.
SOURCE: Current Opinion in Biotechnology, (1996) Vol. 7, No. 2, pp. 181-186. 37 ref. TP248.13.C87
ISSN: 0958-1669
DOCUMENT TYPE: Journal
LANGUAGE: English

L10 ANSWER 8 OF 97 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 96:141156 CABA
DOCUMENT NUMBER: 961609662
TITLE: Visual characterization of recombination at FRT-gusA loci in transgenic tobacco mediated by constitutive expression of the native FLP recombinase
AUTHOR: Bar, M.; Leshem, B.; Gilboa, N.; Gidoni, D.
CORPORATE SOURCE: Department of Plant Genetics, Institute of Field and Garden Crops, ARO, Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.
SOURCE: Theoretical and Applied Genetics, (1996) Vol. 93, No. 3, pp. 407-413. 31 ref. QH431.A1T53
ISSN: 0040-5752
DOCUMENT TYPE: Journal
LANGUAGE: English

L10 ANSWER 11 OF 97 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 95:128398 CABA
DOCUMENT NUMBER: 951607338
TITLE: Cre **recombinase**-mediated site-specific recombination between **plant** chromosomes
AUTHOR: Qin, M.; Bayley, C.; Stockton, T.; Ow, D. W.
CORPORATE SOURCE: Plant Gene Expression Center, US Department of Agriculture, 800 Buchanan Street, Albany, CA 94710, USA.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1994) Vol. 91, No. 5, pp. 1706-1710. 26 ref. Q11.N26
ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

L10 ANSWER 12 OF 97 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 93:105419 CABA
DOCUMENT NUMBER: 931641292
TITLE: Activity of yeast FLP recombinase in maize and rice protoplasts
AUTHOR: Lyznik, L. A.; Mitchell, J. C.; Hirayama, L.; Hodges, T. K.
CORPORATE SOURCE: Department of Botany and Plant Pathology, Purdue University, West Lafayette, IA 47907, USA.
SOURCE: Nucleic Acids Research, (1993) Vol. 21, No. 4, pp. 969-975. 37 ref. QP620.N8
ISSN: 0305-1048

DOCUMENT TYPE: Journal
LANGUAGE: English

L10 ANSWER 13 OF 97 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 93:6430 CABA
DOCUMENT NUMBER: 931634929
TITLE: Gene transfer with subsequent removal of the selection gene from the host genome
AUTHOR: Dale, E. C.; Ow, D. W.
CORPORATE SOURCE: Plant Gene Expression Center, USDA-ARS, 800 Buchanan Street, Albany, CA 94710, USA.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1991) Vol. 88, No. 23, pp. 10558-10562. 39 ref.
ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

QH1.N26

L10 ANSWER 16 OF 97 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 90:133475 CABA
DOCUMENT NUMBER: 901616960
TITLE: Intra- and intermolecular site-specific recombination in **plant** cells mediated by bacteriophage P1 **recombinase**
AUTHOR: Dale, E. C.; Ow, D. W.
CORPORATE SOURCE: Plant Gene Expression Centre, USDA, University of California at Berkeley, Albany, CA 94710, USA.
SOURCE: Gene, (1990) Vol. 91, No. 1, pp. 79-85. 35 ref.
ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English

QH442.G43

L10 ANSWER 22 OF 97 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:725777 CAPLUS
DOCUMENT NUMBER: 133:292010
TITLE: Method for selecting recombinase variants with altered specificity
INVENTOR(S): Sauer, Brian Lee; Rufer, Andreas Walter
PATENT ASSIGNEE(S): Oklahoma Medical Research Foundation, USA
SOURCE: PCT Int. Appl., 144 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2000060091 | A2 | 20001012 | WO 2000-US9154 | 20000406 |
| W: AU, CA, IL, JP, ZA | | | | |
| RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| PRIORITY APPLN. INFO.: | | | US 1999-127977 | 19990406 |

=> d l14 2,4,5,9,19,26 ibib

L14 ANSWER 2 OF 28 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 97:77803 CABA
DOCUMENT NUMBER: 971605696
TITLE: Maize **Activator transposase** has a bipartite DNA binding domain that recognizes

subterminal sequences and the terminal inverted repeats
AUTHOR: Becker, H. A.; Kunze, R.
CORPORATE SOURCE: Institut fur Genetik, Universitat zu Koln, Weyertal 121, 50931 Koln, Germany.
SOURCE: Molecular and General Genetics, (1997) Vol. 254, No. 3, pp. 219-230. 46 ref.
ISSN: 0026-8925
DOCUMENT TYPE: Journal
LANGUAGE: English

L14 ANSWER 4 OF 28 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 95:95690 CABA
DOCUMENT NUMBER: 951605816
TITLE: High level expression of the **Activator transposase** gene inhibits the excision of Dissociation in tobacco cotyledons
AUTHOR: Scofield, S. R.; English, J. J.; Jones, J. D. G.
CORPORATE SOURCE: Sainsbury Laboratory, John Innes Centre, Norwich, NR4 7UH, England, UK.
SOURCE: √ Cell (Cambridge), (1993) Vol. 75, No. 3, pp. 507-517. 44 ref.
ISSN: 0092-8674 QH573.C38
DOCUMENT TYPE: Journal
LANGUAGE: English

L14 ANSWER 5 OF 28 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 95:95649 CABA
DOCUMENT NUMBER: 951605771
TITLE: Fusion of the inducible promoter of the PR-1a gene to the **Activator transposase** gene can transactive excision of a non-autonomous transposable element by external and by internal stimuli
AUTHOR: Charng, Y. C.; Pfitzner, U. M.; Pfitzner, A. J. P.
CORPORATE SOURCE: Botanisches Institut der Ludwig-Maximilians-Universitat Munchen, Menzinger Strasse 67, D-80638 Munchen, Germany.
SOURCE: √ Plant Science (Limerick), (1995) Vol. 106, No. 2, pp. 141-155. 42 ref.
ISSN: 0168-9452 QK1.P69
DOCUMENT TYPE: Journal
LANGUAGE: English

L14 ANSWER 9 OF 28 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:226366 CAPLUS
DOCUMENT NUMBER: 133:130703
TITLE: Intrachromosomal homologous recombination in Arabidopsis induced by a maize transposon
AUTHOR(S): Xiao, Y.-L.; Peterson, T.
CORPORATE SOURCE: Interdepartmental Genetics Program, Department of Zoology and Genetics, Towa State University, Ames, IA, 50010, USA
SOURCE: Mol. Gen. Genet. (2000), 263(1), 22-29
CODEN: MGGEAE; ISSN: 0026-8925
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 61
REFERENCE(S): (1) Athma, P; Genetics 1991, V128, P163 CAPLUS
(2) Bechtold, N; CR Acad Sci 1993, V316, P1194 CAPLUS
(3) Belmaaza, A; Mutat Res 1994, V314, P199 CAPLUS
(4) Biswas, I; J Mol Biol 1998, V279, P795 CAPLUS

(5) Caceres, M; Science 1999 [illegible]285, P415 CAPLUS
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L14 ANSWER 19 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2000:168733 BIOSIS
DOCUMENT NUMBER: PREV200000168733
TITLE: Intrachromosomal homologous recombination in Arabidopsis induced by a maize transposon.
AUTHOR(S): Xiao, Y.-L.; Peterson, T. (1)
CORPORATE SOURCE: (1) Interdepartmental Genetics Program, Department of Zoology and Genetics, Iowa State University, 2206 Molecular Biology Building, Ames, IA, 50010 USA
SOURCE: Molecular and General Genetics., (Feb., 2000) Vol. 263, No. 1, pp. 22-29.
ISSN: 0026-8925.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L14 ANSWER 26 OF 28 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1992:432714 BIOSIS
DOCUMENT NUMBER: BA94:84839
TITLE: HIGH RATES OF AC-DS GERMINAL TRANSPOSITION IN ARABIDOPSIS SUITABLE FOR GENE ISOLATION BY INSERTIONAL MUTAGENESIS.
AUTHOR(S): GREVELDING C; BECKER D; KUNZE R; VON MENGES A; FANTES V; SCHELL J; MASTERSON R
CORPORATE SOURCE: MAX-PLANCK-INSTITUT ZUECHTUNGSFORSCHUNG, CARL VON LINNE WEG 10, D-5000 COLOGNE 30, GER.
SOURCE: PROC NATL ACAD SCI U S A, (1992) 89 (13), 6085-6089.
CODEN: PNASA6. ISSN: 0027-8424.
FILE SEGMENT: BA; OLD
LANGUAGE: English QH.N26

=> d l13 1,2 ibib

L13 ANSWER 1 OF 2 CABA COPYRIGHT 2001 CABI
ACCESSION NUMBER: 93:105419 CABA
DOCUMENT NUMBER: 931641292
TITLE: Activity of yeast FLP **recombinase** in maize and rice protoplasts
AUTHOR: Lyznik, L. A.; Mitchell, J. C.; Hirayama, L.; Hodges, T. K.
CORPORATE SOURCE: Department of Botany and Plant Pathology, Purdue University, West Lafayette, IA 47907, USA.
SOURCE: Nucleic Acids Research, (1993) Vol. 21, No. 4, pp. 969-975. 37 ref.
ISSN: 0305-1048 QP 620.N8
DOCUMENT TYPE: Journal
LANGUAGE: English

L13 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:529275 CAPLUS
DOCUMENT NUMBER: 131:154494
TITLE: Nucleotide sequences encoding maize RAD51 recombinases
INVENTOR(S): Bowen, Benjamin A.; Chamberlin, Mark A.; Drummond, Bruce J.; Mcelver, John A.; Rothstein, Rodney J.
PATENT ASSIGNEE(S): Pioneer Hi-Bred International, Inc., USA
SOURCE: PCT Int. Appl., 66 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9941394 | A1 | 19990819 | WO 1999-US2900 | 19990211 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 9926699 | A1 | 19990830 | AU 1999-26699 | 19990211 |
| EP 1053339 | A1 | 20001122 | EP 1999-906894 | 19990211 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |

PRIORITY APPLN. INFO.: US 1998-74745 19980213
WO 1999-US2900 19990211

REFERENCE COUNT: 3
REFERENCE(S): (1) Smith, K; EMBL Sequence Data Library 1996
(2) William, G; WO 9741228 A 1997 CAPLUS
(3) Yeager Stassen, N; Current Genetics 1996

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